

EFFECT OF ACUTE DIETARY NITRATE SUPPLEMENTATION ON ARTERIAL  
BLOOD OXYGEN SATURATION AND TIME TRIAL PERFORMANCE DURING  
ACUTE HYPOXIC EXPOSURE IN MALE CYCLISTS AND TRIATHLETES

A Thesis  
by  
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## **Abstract**

### **EFFECT OF ACUTE DIETARY NITRATE SUPPLEMENTATION ON ARTERIAL BLOOD OXYGEN SATURATION AND TIME TRIAL PERFORMANCE DURING ACUTE HYPOXIC EXPOSURE IN MALE CYCLISTS AND TRIATHLETES.**

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Previous investigations of high altitude exercise have demonstrated that hypoxia induced pulmonary vasoconstriction can be reduced and blood oxygen saturation levels and exercise performance can be partially restored through the use of pulmonary vasodilators such as phosphodiesterase (PDE-5) inhibitors. Similarly, nitric oxide supplementation via exogenous nitric oxide and nitrite has been reported to cause pulmonary vasodilation; however, exogenous dietary nitrate, such as red beetroot juice, has only been tested and shown to cause vasodilation in the systemic circulation and has only been tested and shown to increase athletic aerobic exercise performance at normoxia, sea level. **PURPOSE:** To determine the effect of short-term red beetroot juice supplementation on systemic blood pressure, exercising arterial blood oxygen saturation, and time trial performance during acute hypoxic exposure ( $F_{I}O_2 \sim 13\%$ ). **METHODS:** Nine healthy competitive male cyclists and triathletes were randomly assigned in a crossover design to perform one time trial on a cycle ergometer while experiencing each of the three acute supplemental treatments: beetroot juice (BR), nitrate depleted beetroot juice (PL), and water (CO), 2.5 hours before each experimental time trial. Systemic blood pressure was measured before the supplement was

ingested and pre and post- warm-up after the supplement was ingested. Oxygen saturation, completion time, and work rate were measured after approximately every 1/4 total kilojoules completed in addition to the total time trial completion time, work rate, cycling economy, and oxygen consumption for each experimental trial. RESULTS: No significant main effects due to treatment were observed in systolic blood pressure (BR vs PL,  $P=0.24$ ; BR vs CO,  $P=0.35$ ; PL vs CO,  $P=0.95$ ), diastolic blood pressure (BR vs PL,  $P=0.99$ ; BR vs CO,  $P=0.99$ ; PL vs CO,  $P=0.98$ ), and mean arterial pressure (BR vs PL,  $P=0.96$ ; BR vs CO,  $P=0.62$ ; PL vs CO,  $P>0.99$ ). No significant differences in time trial completion times (BR vs PL,  $P=0.33$ ; BR vs CO,  $P=0.87$ ; PL vs CO,  $P=0.50$ ) (Mean  $\pm$  SD: BR,  $911.78 \pm 163.07$  sec; PL,  $865.78 \pm 131.02$  sec; and CO,  $888.44 \pm 142.32$ ) or work rates (BR vs PL,  $P=0.27$ ; BR vs CO,  $P=0.91$ ; PL vs CO,  $P=0.33$ ) were observed when comparing the three treatments across total and segment results. No significant differences were observed in oxygen saturation at each completion point during the time trial (BR vs PL,  $P=0.69$ ; BR vs CO,  $P>0.99$ ; PL vs CO,  $P=0.83$ ). Although no treatment effects were observed, time effects were observed in certain variables. Finally, there were no significant treatment effects on oxygen consumption (BR vs PL,  $P=0.99$ ; BR vs CO,  $P=0.99$ ; PL vs CO,  $P=0.99$ ) or mean cycling economy (Work Rate / Oxygen Consumption) ( $J \cdot \text{min}^{-1} / \text{ml} \cdot \text{min}^{-1}$ ) during the time trial (BR vs PL,  $P=0.90$ ; BR vs CO,  $P>0.99$ ; PL vs CO,  $P=0.77$ ). CONCLUSION: Acute beetroot juice supplementation did not affect systemic blood pressure, exercising arterial blood oxygen desaturation, and time trial cycling performance during acute hypoxic exposure. Therefore, beetroot juice may not be a prospective aid to help improve hypoxic aerobic exercise performance.

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## **Introduction**

As altitude increases, the inspired partial pressure of oxygen ( $P_{iO_2}$ ) decreases (Hahn & Gore, 2001). This decrease in  $P_{iO_2}$  reduces the available oxygen to the body (also known as hypoxia), and impairs the aerobic system, hindering optimal cycling performance, even in short events such as the 4000 m pursuit (Hahn & Gore, 2001). In addition to overall slower winning times for events (Martin, Levett, Grocott, & Montgomery, 2010), power output (work performed/time) has also been recorded to be lower at high altitude than those outputs at sea level for the same work intervals completed by cyclists (Hahn & Gore, 2001). The level of impairment of the aerobic system is relative to the degree of altitude, intensity of exercise (Hahn & Gore, 2001), and training and acclimatization status of the athlete (Martin, Levett, Grocott, & Montgomery, 2010). The physiological responses to aerobic exercise at hypoxia become exacerbated and the aerobic performance becomes more impaired as altitude increases (Bartsch & Saltin, 2008). The degree of altitude can be categorized as low (500-2000 m), moderate (2000-3000 m), high (3000-5500 m), and extreme (above 5500 m) (Bartsch & Saltin, 2008). High altitude can be further defined as the “threshold...where there are noticeable physiological changes in response to the reduction in oxygen availability” (Martin et al., 2010, p.464), which can not be restored with acclimatization to a healthy response at sea level (Bartsch & Saltin, 2008); therefore, supplements that will reduce hypoxic pulmonary vasoconstriction may be of interest in improving performance and in making athletes more competitive at high altitude.

## **Background Physiology**

With exercise at sea level, the body increases ventilation (Hahn & Gore, 2001), cardiac output, and blood flow to supply oxygen and substrates to support the increased metabolic rate of working muscle (Sheel, MacNutt, & Querido, 2010). High altitude complicates and limits this process of oxygen transport during exercise. In addition to reduced oxygen availability, at altitude above 2500 m (moderate altitude), hypoxia causes a reflex mechanism of constricting pulmonary blood vessels in areas of lower oxygen content within minutes (Martin et al., 2010). This increase in pulmonary vascular resistance decreases the gas exchange interface between the lungs and blood (Blitzer, Loh, Roddy, Stamler, & Creager, 1996; Sheel et al., 2010), reduces pulmonary capillary blood transit times, and increases pulmonary arterial pressure (Martin et al., 2010; Sheel et al., 2010). These diffusion limitations lead to reduced arterial blood oxygen content. In addition, the increase in pulmonary arterial pressure has been linked to pulmonary edema (fluid leaking out of the blood into the tissue) that further limits oxygen transfer from the lungs to the blood by increasing the area across which oxygen has to diffuse (Scherrer et al., 1996). Furthermore, the increased resistance exacerbates the load of which the right ventricle has to overcome to eject blood, resulting in a smaller amount of blood ejected (stroke volume) and less oxygen available to the body (Hsu et al., 2006). The decreased oxygen availability is detrimental to aerobic exercise performance (Sawka, Convertino, Eichner, Schnieder, & Young, 2000).

The body has several mechanisms to compensate for this decrease in oxygen availability and hypoxic pulmonary vasoconstriction. The body reacts both acutely (within minutes) via hyperventilation (Martin et al., 2010), increased heart rate, and increased cardiac

output (Hahn & Gore, 2001), and chronically (within hours and days) via adaptations such as increased red blood cell volume / total blood volume percentage (Sawka et al., 2000), red blood cell production (Hahn & Gore, 2001), and alterations in skeletal muscle substrate utilization and metabolism (Hahn & Gore, 2001; Martin et al., 2010). In addition, the systemic vasculature undergoes vasodilation in response to hypoxia, which increases blood flow better to support the oxygen demand of working tissue (Blitzer et al., 1996). While these physiological changes do increase the oxygen available to the muscles, reducing the deficit in aerobic performance (Hahn & Gore, 2001), they are only able partially to compensate for the strain of high altitude or hypoxia (Bartsch & Saltin, 2008; Hahn & Gore, 2001; Martin et al., 2010; Sawka et al., 2000; Sheel et al., 2010). The end result is that high altitude performance is never able to reach that of sea-level or normoxic conditions (Martin et al., 2010).

### **Proposed Mechanism of Improvement**

The decrease in arterial blood oxygen saturation observed at high altitude with increasing exercise is a result of the mismatch of lung ventilation to pulmonary blood flow (ventilation-perfusion mismatch) and a reduced gas exchange due to diffusion limitations and a reduced oxygen availability (Schoene, 2001). Furthermore, Hahn & Gore (2001) and Blitzer et al. (1996) concluded that tissue oxygen extraction is very efficient and that systemic circulation reacts to hypoxia by increasing oxygen delivery to better support sub-maximal aerobic performance at high altitude, respectively. For those reasons, the on-loading of oxygen at the lung and pulmonary blood oxygen delivery should be examined as a possible route to improve performance.

Specifically, pulmonary vasodilation can reduce hypoxia-induced arterial blood oxygen desaturation and increase oxygen delivery by increasing ventilation-perfusion matching and decreasing pulmonary arterial pressure and pulmonary vascular resistance, thus reducing the diffusion limitation (Scherrer et al., 1996; Zuckerbraun, George, & Gladwin, 2011) and load on the heart (Hsu et al., 2006). The use of pulmonary vasodilators has been shown to assist in reducing hypoxia-induced pulmonary vasoconstriction by decreasing pulmonary arterial pressure and chance of edema, re-routing more blood flow to better ventilated areas, increasing pulmonary-capillary blood transit time, and increasing the pulmonary capillary interface, allowing more efficient and effective oxygen exchange from the lung tissue to the blood (Scherrer et al., 1996). A decrease in pulmonary vascular resistance has also been shown to reduce the increased pressure and load on the heart and result in a larger circulation of blood (and oxygen) to compensate for and support the physiological stress associated with high altitude (Gotshall, 2007). In conclusion, pulmonary vasodilation enhances oxygen transfer between the lung and blood by minimizing the ventilation-perfusion mismatch and diffusion limitation while reducing the strain on the cardiovascular system from high altitude to increase the oxygen available to the body and thus better preserve exercise performance.

### **The Effects of Nitric Oxide**

While the body does have a system that naturally responds to the stimulus of hypoxia with a mechanism of vasodilation in the systemic circulation, animal experiments show that this mechanism might not be as functional in the pulmonary circulation to counter or reduce the hypoxic vasoconstriction reflex (Blitzer et al., 1996). Moreover, this compensatory

mechanism seems to be impaired in the pulmonary system in severe hypoxia (Blitzer et al., 1996); therefore, additional assistance with vasodilation may be helpful and necessary in preserving performance in hypoxic conditions. The body naturally converts L-arginine to nitric oxide via nitric oxide synthase (Zuckerbraun et al., 2011). Endothelial derived nitric oxide stimulates production of cyclic guanosine monophosphate, a second messenger responsible for signaling smooth muscle relaxation (Zuckerbraun et al., 2011), to induce vasodilation and reduce vascular resistance and pressure (Blitzer et al., 1996). Furthermore, it has been supported that under acidic and hypoxic environments, such as those created during exercise and high altitude, respectively, this reaction can be enhanced (Rassaf et al., 2007). Rassaf et al. (2007) reported that the oxidation product of nitric oxide, plasma nitrite, in addition to age “predicts the maximal stress achieved and duration of exercise” (p.672). Not only is increased nitrite and therefore nitric oxide production related to hypoxia and vasodilation, but it also is related to increased exercise intensity and performance.

## **Supplementation**

In addition to endogenous nitric oxide production, supplemental nitric oxide sources in the form of nebulized nitrite, infused sodium nitrite, and inhaled nitric oxide have been shown to increase pulmonary vasodilation in hypoxic environments resulting in decreased pulmonary vascular resistance and pulmonary arterial pressure in both humans and animals (Bailey et al., 2009; Ingram, Pinder, Bailey, Fraser, & James, 2010; Modin et al., 2001; Scherrer et al., 1996; Zuckerbraun et al., 2011; Zuckerbraun et al., 2010). Inhaled nitric oxide has even been shown to help reduce pulmonary hypertension (high blood pressure) and re-route blood flow away from areas of high edema to areas of low edema (Scherrer et al.,



1996). This increases oxygen saturation by reducing the ventilation perfusion mismatch and the difference in oxygen content between the lungs and blood (improving the diffusion limitation) in people with high altitude induced pulmonary edema (Scherrer et al., 1996). In conclusion, exogenous sources of nitrite such as infused sodium nitrite and inhaled nebulized nitrite and nitric oxide have been reported to induce pulmonary vasodilation and reduce hypoxic pulmonary vasoconstriction while endogenous nitrite and nitric oxide levels have been linked to exercise performance.

Another source of exogenous nitrate, dietary nitrate, is converted from nitrate to nitrite by bacteria-producing enzymes in saliva and the oral cavity, which then react with tissue specific enzymes to produce nitric oxide (Zuckerbraun et al., 2011). Due to the invasiveness of pulmonary vascular resistance measurements, much of this research is based on animal models or the use of expensive indirect measures estimating pulmonary vascular resistance in humans (Gotshall, 2007; Ingram et al., 2010); therefore, most of the studies on dietary nitrate supplementation with humans only examined systemic vascular pressures and not pulmonary vascular pressures. Studies have shown that dietary nitrates do have vasodilatory and blood pressure reduction properties when examining systemic vasculature in both humans (Webb et al., 2008) and primates (Kapil et al., 2010). These experiments support that the nitrate ingredient reduces systemic blood pressure by initiating vasodilation via conversion of nitrate to nitrite to nitric oxide.

Along with reducing systemic blood pressure (Vanhatalo et al., 2010), dietary nitrates have been linked to increases in exercise performance in humans (Bailey et al., 2009; Gotshall, 2007; Lansley et al., 2011; Vanhatalo et al., 2010). Red beetroot, the dietary source used in the previous studies, is a vegetable with high nitrate content (Hord, Tang, & Bryan,

2009; Tamme et al., 2010). Dietary nitrate supplementation via red beetroot juice has been reported to reduce oxygen cost during sub-maximal intensity exercise (Bailey et al., 2009; Vanhatalo et al., 2010), to improve high intensity exercise tolerance (Bailey et al., 2009; Vanhatalo et al., 2010), to improve time to exhaustion and peak power output during a ramp incremental to maximum test (Vanhatalo et al., 2010), and to improve cycling economy (power output/ work rate) and time trial performance (Lansley et al., 2011). In summary, results from these experiments demonstrate that the nitrates in beetroot juice have a positive effect on aerobic performance.

Overall, dietary nitrates via beetroot juice have been shown to initiate systemic vasodilation (Kapil et al., 2010; Webb et al., 2008) and to increase aerobic exercise performance at normoxia (sea level) (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010); however, these studies that analyzed the effects of nitrate rich beetroot juice only examined blood pressure and vascular resistance in the systemic system, not the pulmonary system. They also only examined exercise performance at normoxia. It is unknown if the nitrates in beetroot juice have effects on pulmonary vasculature and aerobic exercise performance at hypoxia. Meanwhile, Gotshall (2007) concluded that phosphodiesterase 5 (PDE-5) inhibitors, which work downstream to nitrates in the same vasodilatory pathway, reduce hypoxia-induced pulmonary vasoconstriction and improve exercise performance at high altitude or during hypoxia more than the body's own compensatory mechanisms alone (Gotshall, 2007). PDE-5 inhibitors were determined to increase arterial blood oxygen saturation and blood oxygen delivery (Ghofrani et al., 2004; Hsu et al., 2006; Richalet et al., 2005) and lead to an increase in work capacity and thus aerobic exercise performance during hypoxic exposure (Ghofrani et al., 2004; Hsu et al., 2006; Richalet et al., 2005). In addition,

PDE-5 inhibitors have specific effects on the pulmonary system but do not reduce blood pressure in the systemic system at hypoxic (Gotshall, 2007). This evidence demonstrates that the reduction of pulmonary vascular resistance is related to increases in oxygen availability to the body and increases in high altitude/ hypoxic performance.

### **Statement of the Problem**

Previous studies have shown that pulmonary vasodilation increases arterial blood oxygen saturation and blood oxygen delivery (Ghofrani et al., 2004; Hsu et al., 2006; Ricart et al., 2005; Richalet et al., 2005) and leads to increases in aerobic exercise performance at high altitude and hypoxia (Ghofrani et al., 2004; Hsu et al., 2006; Richalet et al., 2005). While it is known that nitric oxide causes pulmonary vasodilation (Ingram et al., 2010; Scherrer et al., 1996; Zuckerbraun et al., 2011), sources of dietary nitrates, such as red beetroot juice, have only been examined in affecting systemic circulation (Kapil et al., 2010; Webb et al., 2008). Furthermore, this systemic mechanism to improve aerobic performance has only been tested at normoxia (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010). It is unknown if dietary nitrates can increase arterial blood oxygen saturation that is reduced due to hypoxia-induced pulmonary vasoconstriction and lead to an increase in high altitude/hypoxic acute exercise performance. The purpose of this study was to determine the effect of short-term red beetroot juice supplementation on systemic blood pressure, exercising arterial blood oxygen saturation, and acute aerobic (approximately 200 kJ) time trial performance during acute hypoxic exposure (simulated high altitude (4300 m)) in male club level cyclists and triathletes.

## **Hypothesis**

Pulmonary vasodilation results in increased oxygen availability via increased blood oxygen content and increased blood oxygen delivery to help support aerobic performance (Gotshall, 2007), so an increase in blood oxygen saturation and blood volume ejected from the heart would suggest the occurrence of pulmonary vasodilation. Because nitric oxide causes pulmonary vasodilation in hypoxia and red beetroot juice and pulmonary vasodilation have both been related to increases in aerobic performance, we hypothesized that acute supplementation of red beetroot juice, which is high in nitrate content, would lead to a decrease in systemic blood pressure, an increase in exercising arterial blood oxygen saturation, and a decrease in cycling time trial performance time during acute hypoxic exposure in male club level cyclists and triathletes.

## **Significance of the Study**

Beetroot juice could potentially reduce the deficit in aerobic exercise performance seen at high altitude when compared to sea level. This is important because many sporting events are held at moderate to high altitude, where past aerobic performances were markedly affected (Martin et al., 2010). Furthermore, evidence of this mechanism could be very significant and beneficial to multiple populations. Not only could it help non-acclimatized athletes be more competitive at high altitude events, but it could also help military personnel and other workers perform duties better and more efficiently that would otherwise be limited in high altitude or hypoxic environments. In addition, dietary nitrate induced pulmonary vasodilation may be a prospective aid for reducing pulmonary hypertension, hypoxia, and pulmonary edema, ultimately improving everyday performance in diseased populations

(Zuckerbraun et al., 2011). While dietary nitrate, such as red beetroot juice, is a natural vasodilator with few possible minor side effects at this acute dose (Bailey et al., 2009; Gilchrist, Winyard, & Benjamin, 2010) compared to other vasodilators such as PDE-5 inhibitors (Gotshall, 2007), dietary nitrate supplementation is also more practical during performance and everyday activity since one can easily supplement without being restricted by inhalation and infusion mechanisms; therefore, it was beneficial to investigate the pulmonary vasodilatory potential of dietary nitrate in relation to hypoxic environments and exercise performance.

## Definition of Terms

Acclimatization: a physiological process of adaption to an environmental stressor such as hypoxia

Adenosine Triphosphate (ATP): a cellular energy transporter

Afterload: the pressure or force in which the heart has to pump against to eject blood

Alveoli: terminal gas exchanging tissue of the lung

Aerobic capacity: the maximum amount of work that can be performed due the body's rate of consuming and using oxygen to make energy

Ambient barometric pressure: the total atmospheric pressure specific to altitude

Artery (arterial): blood vessels leaving the heart carrying oxygenated blood such as the aortic artery leaving the heart; pulmonary arteries: blood vessels bringing deoxygenated blood from the peripheral circulation to become oxygenated at the lungs

Capillary: terminal gas exchanging tissue of blood vessels

Cardiac output: the amount of blood pumped per minute

Chemiluminescence technique: a technique used for detection of various molecules and substance in a medium such as plasma nitrite

Diffusion limitations: Impaired movement of molecules or substances (oxygen) from areas of high concentration to areas of low concentration.

Diastolic blood pressure: the lowest pressure in the arteries; when the heart relaxes

Echocardiography: non-invasive test that uses sound waves to create pictures of the heart; Doppler echocardiography: assesses cardiac tissue and blood velocity used to examine vascular and cardiac function such as pulmonary arterial blood pressure and cardiac output

Edema: fluid that leaks out the blood vessels into the tissue

Ejection fraction: the fraction of blood ejected from the heart compared to the total blood volume in the heart; measure of stroke volume as a percentage of total blood volume in the heart

Fraction of inspired oxygen ( $F_{I}O_2$ ): the percentage/proportion of total inspired air in the lungs that is composed of oxygen

Gas exchange threshold: the breakpoint during exercise in which carbon dioxide gas production increases disproportionally to the amount of oxygen consumed; a non-invasive estimate for anaerobic threshold: the point where the body has to rely more on energy systems that do not use oxygen to supply the energy need to support exercise (since the energy rate required exceeds the supply rate capable of the aerobic system which uses oxygen for energy production)

Gas rebreathing technique: non-invasive procedure used to determine cardiac output via gas analysis after inspiration of two marker compounds

High altitude: 3000 – 5500 m; the “threshold where there are [significant] noticeable physiological changes in response to the reduction in oxygen availability” which can not be restored with acclimatization to a healthy response at sea level (Bartsch & Saltin, 2008)

Hypoxia: the reduction in oxygen available

Kinetics: the movement of a molecule or substance

Low altitude: 500 – 2000 m (Bartsch & Saltin, 2008)

Moderate altitude: 2000 – 3000 m (Bartsch & Saltin, 2008)

Normoxia: sea level conditions

Partial pressure of inspired oxygen ( $P_{I}O_2$ ): the pressure due to oxygen (amount of oxygen) in the inspired gas that reaches the lungs

Phosphodiesterase 5 inhibitors (PDE-5 inhibitors): oral pharmacological supplemental pulmonary vasodilators

Pulmonary circulation: vasculature leaving the heart to become oxygenated at the lung and leaving the lung once it is oxygenated to be pumped to the periphery by the heart.

Pulmonary Hypertension: high blood pressure in the pulmonary vasculature

Pulmonary Vasoconstriction: the reduction of the diameter of pulmonary blood vessels

Pulse oximeter: non-invasive instrument used to measure oxygen saturation and heart rate

Sphygmomanometer: an instrument that uses an inflatable cuff to restrict blood flow in order to measure blood pressure

Strain gauge plethysmography: technique used to determine blood flow through an artery to examine the dilatory properties of the artery in reaction to various stimuli

Stroke volume: the amount of blood pumped per heart contraction

Systemic circulation: peripheral vasculature (all vessels excluding the heart and pulmonary vasculature)

Systolic blood pressure: the highest pressure in the arteries; when the heart contracts

Vasodilation: increased diameter of blood vessels

Ventilation-Perfusion matching: the matching of well ventilated alveoli (lung tissue) with well perfused pulmonary capillaries

Work capacity: the maximum possible amount of work (force/distance) produced



## **Review of Literature**

Athletic performance is impaired at high altitude due to a decreased oxygen availability to the body to support aerobic exercise (Hahn & Gore, 2001; Martin et al., 2010; Scherrer et al., 1996; Schoene, 2001; Sheel et al., 2010). Not only is the inspired partial pressure of oxygen ( $P_{iO_2}$ ) reduced as altitude increases (Hahn & Gore, 2001), but the available oxygen is further limited by the hypoxia-induced reflex of pulmonary vasoconstriction within minutes of exposure to altitude above 2500 m (Martin et al., 2010). Pulmonary vasoconstriction reduces both the oxygen exchanged from the lung to the blood (reducing arterial blood oxygen saturation) and the blood oxygen delivered from the heart to the tissue; however, supplemental pulmonary vasodilation has been shown to attenuate hypoxic pulmonary vasoconstriction (Gotshall, 2007). Supplemental pulmonary vasodilators also have shown potential of improving high altitude aerobic performance (Gotshall, 2007). While inhaled nitric oxide and infused and nebulized nitrite have been shown to cause pulmonary vasodilation in hypoxia (Ingram et al., 2010; Scherrer et al., 1996; Zuckerbraun et al., 2011), sources of dietary nitrate, such as red beetroot juice, have only been examined and shown to cause vasodilation in the systemic vasculature in normoxia (Kapil et al., 2010; Webb et al., 2008). Dietary nitrate via red beetroot juice has also only been tested and shown to increase aerobic exercise performance in normoxia (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010).

This review of literature examines previous research on: pulmonary vasodilation via phosphodiesterase 5 (PDE-5) inhibitors (Sildenafil) to reduce hypoxic pulmonary

vasoconstriction and increase high altitude/hypoxic performance, the vasodilatory effects of three sources of nitric oxide, and the effects of nitrates on performance at normoxia (sea level). Along with examining this research, this knowledge was used to set up an experiment to fill in the gaps via connecting the three areas of interest: nitrate supplementation, pulmonary vasodilation, and high altitude/hypoxic aerobic exercise performance. It is unknown if acute supplementation of dietary nitrates can decrease arterial blood oxygen desaturation that results from hypoxia-induced pulmonary vasoconstriction and can lead to an increase in hypoxic/high altitude acute aerobic performance. The purpose of this study was to determine the effect of short-term dietary nitrate supplementation via red beetroot juice on systemic blood pressure, exercising arterial blood oxygen saturation, and acute aerobic (approximately 200 kJ) time trial performance during acute hypoxic exposure (simulated high altitude (4300 m)) in male club-level cyclists and triathletes.

### **Pulmonary Vasodilators Improve Performance**

Phosphodiesterase 5 (PDE-5) inhibitors are vasodilators that have previously been effective in treating pulmonary hypertension and are now being examined as a possible oral supplement to attenuate hypoxic pulmonary vasoconstriction during high altitude exposure (Gotshall, 2007). PDE-5 inhibitors work downstream to nitrates in the same nitric oxide induced vasodilation pathway by increasing the activity of second messenger cyclic guanosine monophosphate (cGMP) ultimately leading to enhanced vasodilatory effects (Gotshall, 2007). Four studies (Ghofrani et al., 2004; Hsu et al., 2006; Ricart et al., 2005; Richalet et al., 2005) have explored Sildenafil, an oral PDE-5 inhibitor, in relation to both pulmonary vasodilation and high altitude or hypoxic exercise performance. Even though

each study used a slightly different procedure (hypoxic environment and exercise performance test), they found overall similar results just of different magnitudes which correlated with their specific parameters (degree of hypoxic environments, length of exposure, and type and intensity of exercise test). In conclusion, Sildenafil significantly reduced pulmonary arterial pressure, increased oxygen saturation, increased cardiac output, and increased work rate (Ghofrani et al., 2004; Hsu et al., 2006; Ricart et al., 2005; Richalet et al., 2005).

**Pulmonary vasodilation.** Supplements are needed to attenuate the increased pulmonary vascular resistance characteristic to hypoxic environments in order to increase the oxygen available to working tissue (Gotshall, 2007). Ricart et al. (2005) found that Sildenafil significantly decreased pulmonary arterial pressure during rest and exercise in hypoxia only, not in normoxia, suggesting that PDE-5 inhibitors work through relieving the pulmonary vasoconstriction only present in hypoxic situations. Due to the invasiveness of heart catheterization to measure pulmonary arterial pressure directly to examine pulmonary vascular resistance, the three studies that examined the pulmonary vasculature, used echocardiography to measure pulmonary arterial pressure indirectly (Ghofrani et al., 2004; Ricart et al., 2005; Richalet et al., 2005); however, this technique is very hard to perform during exercise, and the results can be further skewed by the heart health of the subject and experience of the operator (Hsu et al., 2006). Consequently, these results should only be examined in addition to other measures to draw a conclusion about pulmonary vascular resistance (Hsu et al., 2006). Furthermore, there were slight differences in the protocol as to the time the pressure measurement was taken (right after exercise as in Ricart et al.'s study

(2005), during exercise as in Ghofrani et al.'s study (2004), or at rest post-exercise in Richalet et al.'s study (2005)), which results in some variability in the data. Along with measuring pulmonary arterial pressure, these experimenters also analyzed the presence of pulmonary vasoconstriction with other measures. Hypoxic pulmonary vasoconstriction increases the pulmonary arterial pressure by increasing vascular resistance, decreases the gas exchanged at the lung by creating diffusion limitations, and decreases the blood ejected from the heart by increasing the load on the right ventricle (Hsu et al., 2006). In addition to a decrease in pulmonary arterial pressure, increases in oxygen exchanged (blood oxygen saturation) and blood ejected (stroke volume SV) would suggest that vasodilation helped attenuate this constriction.

***Oxygen saturation.*** While blood oxygen saturation did increase with the administration of Sildenafil compared to no supplementation at hypoxia in all the studies (Ghofrani et al., 2004; Hsu et al., 2006; Ricart et al., 2005; Richalet et al., 2005), the results varied due to the different exercise testing procedures, different hypoxic environment conditions, and individual subject variability. While all four experiments studied aerobic exercise using a cycle ergometer, Ricart et al. (2005) and Hsu et al. (2006) both used constant work rate bouts of five minutes at 50% of a theoretical  $\text{VO}_2\text{max}$  specific for the subject and thirty minutes at 55% of peak power output followed by a 6 km time trial, respectively. They both recorded slightly lower improvements in oxygen saturation with the supplementation of Sildenafil than Richalet et al. (2005) and Ghofrani et al. (2004), who used incremental exercise to exhaustion bouts. This is due to the intensity of exercise. When exercise intensity increases, the metabolic rate of working tissue and therefore the physiological

stresses on the body increase to support the more intense exercise (Martin et al., 2010). The body requires an increased amount of oxygen to support this larger magnitude of stress; however, when oxygen is limited such as at hypoxia, one's physiological response to exercise is further impaired due to the increased deficit in oxygen required to meet the demands of the higher exercise intensity (Sheel et al., 2010). Because larger oxygen deficits are seen with higher intensity exercise, improvements in blood oxygen saturation levels would result in more substantial performance and physiological improvements than those less impaired at lower intensities. The body is also impaired to a higher extent as altitude increases because less oxygen is available at higher altitudes (Bartsch & Saltin, 2008; Hahn & Gore, 2001). This correlation is evident in the Hsu et al. (2006) and Ghofrani et al. (2004) studies. When comparing both results of acute hypoxic exposure, Hsu et al. (2006) reported lower improvements with Sildenafil than Ghofrani et al. (2004), who tested at a higher degree of hypoxia. Ricart et al. (2005) received a low value of oxygen saturation improvement with Sildenafil for their degree of hypoxia. In addition to a shorter exercise bout and hypoxic exposure, their exercise protocol called for measurements taken post-exercise and not at the end of exercise, thus leading to an underestimate of change in oxygen saturation (Ricart et al., 2005). Finally, the length of exposure in the hypoxic environment led to variance in the results. For these experiments, acute exposure can be defined as less than two hours while chronic exposure can be defined as six days. Ghofrani et al. (2004) used a double blind crossover study (to rule out most inter-individual variability) in which each athlete was exposed to both the placebo and Sildenafil treatments at both an acute hypoxic gas environment for two hours and a chronic six day hypoxic exposure at the actual corresponding altitude. Sildenafil was reported to help oxygen saturation during the acute

hypoxic exposure but did not seem to help during the short term chronic high altitude exposure (Ghofrani et al., 2004). Ghofrani et al. (2004) suggested that this difference could be due to short term exposure acclimatization effects that help improved ventilation-perfusion matching and gas exchange during the six day exposure. They concluded that Sildenafil just maintained the improved or slightly improved oxygenation in the chronic hypoxic environment (Ghofrani et al., 2004). These acclimatization effects had not yet developed during the acute two-hour hypoxic exposure (Ghofrani et al., 2004).

Several adaptations take place during short term acclimatization to help oxygen uptake from the lung. Ventilation continues to increase as the peripheral chemoreceptors become more sensitive to hypoxia (Bartsch & Saltin, 2008). This increased ventilation of the lung tissue enhances gas exchange which increases the oxygen content of the blood (Sawka et al., 2000). Increased ventilation is a significant factor in enhancing blood oxygen saturation during the first two weeks of hypoxic exposure (Bartsch & Saltin, 2008). Therefore, Sildenafil would only help to maintain the improved or slightly improve oxygen saturation by reducing the constriction of the pulmonary tissue and increasing the area available for gas exchange. As a result, it would seem less effective in increasing oxygen saturation in chronic hypoxic environments than acute hypoxic environments since adaptations have occurred in the chronic environments to offset partially the gas exchange impairment.

Furthermore, chronic hypoxic exposure activates mechanisms that may reduce blood oxygen saturation measurements to allow for more efficient usage of available substrates or to increase the oxygen available to the muscle. There are some adaptations in the skeletal muscle with more prolonged exposure to hypoxia such as altered substrate usage and

metabolism to decrease the reliance on and demand of oxygen (Hahn & Gore, 2001; Martin et al., 2010), which could skew the magnitude of the change in oxygen saturation. In addition, within a few hours of high altitude exposure, 2,3 diphosphoglycerate concentration is elevated, which assists red blood cells with unloading oxygen from the blood to the muscles (Hahn & Gore, 2001). This increased unloading at the muscle may cause the blood that is returning to the lungs to contain a lower oxygen saturation level so that once it becomes oxygenated, the level is not as high as it was originally since it had to overcome this deficit in saturation (Hahn & Gore, 2001). This would skew the arterial blood oxygen saturation measurement making Sildenafil seem like it is not as effective in increasing oxygen saturation in the chronic hypoxic environment compared to acute hypoxic exposure. The muscle may be unloading some of that extra oxygen delivered due to Sildenafil supplementation and using it to increase exercise performance. Hahn & Gore (2001) concluded that oxygen extraction at the muscle was highly efficient during acute altitude exposure, when referencing cyclists, which suggests on-loading oxygen and getting it to the muscle may be more of a significant factor to target for improvement during high altitude exercise.

Additionally, chronically increased ventilation causes respiratory alkalosis (Bartsch & Saltin, 2008). In return, the kidney excretes bicarbonate to help the acid-base balance (Bartsch & Saltin, 2008). This consequently reduces the body's acid-base buffering capacity (Bartsch & Saltin, 2008). Furthermore, respiratory alkalosis manifests several days after exposure (Bartsch & Saltin, 2008). Respiratory alkalosis is maintained for several days before reversing towards basal levels (Bartsch & Saltin, 2008). The rate and magnitude of this reversal is negatively correlated to elevation (Bartsch & Saltin, 2008). Meanwhile, the

reduced buffering capacity leaves the body more vulnerable to the reduction in pH brought about by exercise ultimately resulting in a decrease in performance (Bartsch & Saltin, 2008). This reduction in pH has been shown to impair and slow the movement of oxygen (Sheel et al., 2010) from the lung to the blood and muscle. This may reduce the effectiveness of Sildenafil in increasing blood oxygen saturation; however, this does not mean it is less effective in inducing vasodilation. Pulmonary arterial pressure decreased in both acute and chronic environments while cardiac output (the amount of blood pumped per time) increased (Ghofrani et al., 2004), suggesting Sildenafil was effective in creating vasodilation in both environments.

Since one way pulmonary vasodilation helps to increase the oxygen available is by increasing gas exchange (Ghofrani et al., 2004; Hsu et al., 2006), an increase in oxygen saturation would suggest the occurrence of pulmonary vasodilation. The measurement of oxygen saturation to determine the presence of pulmonary vasodilation seems to be confounded by acclimatization factors in chronic hypoxic environments; therefore, this experiment used an acute hypoxic environment to determine if pulmonary vasodilation is attenuating pulmonary vasoconstriction by improving gas exchange and thus oxygen saturation. Due to lack of access to expensive echocardiography instruments to measure indirectly pulmonary arterial pressure such as the Doppler system, and the invasiveness of the more accurate direct pulmonary vascular resistance measures such as a catheter, blood oxygen saturation was measured as evidence to suggest the presence of pulmonary vasodilation. Because an increase in arterial blood oxygen saturation is one possible result of the reduction of pulmonary vasoconstriction, arterial blood oxygen saturation can give the investigator an idea of the degree of pulmonary vasculature tone. Since a hypobaric chamber



or high altitude testing facility was not accessible or feasible for this experiment, an acute gas exposure was used. The hypoxic environment mimicked that of 4300 m, similar to Richalet et al.'s study, with a corresponding 12.6-12.7% fraction of inspired oxygen ( $F_{I}O_2$ ) gas compared to the normal 20.93% (West, 1996). Furthermore, this is the altitude of Pike's Peak in Colorado (West, 1996), which is a potential testing facility for further application and investigation.

Even though a high altitude environment would be more realistic and applicable to athletes who have to ascend several days before a race and therefore undergo these short acclimatization effects, the acute exposure is still fairly applicable. Sawka et al. (2000) determined that the contribution of hemoconcentration due to erythropoiesis to the increase in oxygen content at 4300 m is much less significant than the contribution by ventilatory acclimatization, which raises alveolar ventilation to increase gas exchange from the lung to the blood thus raising blood oxygen content. This shows that improving mechanisms that interfere with gas exchange from the lung to the blood would be more significant in helping oxygen saturation at 4300 m than improving the blood's oxygen carrying capacity. This experimental test of pulmonary vasodilation in the acute setting is applicable to real life situations because it targets the same more substantial limiting factor (gas exchange) that ventilatory acclimatization does in chronic hypoxic exposure. Vasodilation works to increase the interface of the alveolar membrane and pulmonary capillaries, reducing the pulmonary capillary transit time, and by matching ventilation with perfusion to accomplish the same goal of increased gas exchange. Furthermore, the purpose was to study an acute bout of exercise (similar to that in all four studies) to simulate a race, not the chronic effects of training at high altitude. During a shorter exposure, the situation is not further confounded

with more long term adaptations such as the increase in red blood cell mass that takes several weeks (Hahn & Gore, 2001). This longer exposure would also not be applicable to athletes that ascend just a few days before a race and are not interested in training or able to train at high altitude. This evidence suggests that pulmonary vasodilation, in an acute setting, would be validly measured by an increase in oxygen saturation (evidence of on-loading more oxygen) and applicable to a more realistic situation where an athlete only resides at high altitude for several days just to compete in a race.

***Blood oxygen delivery.*** Pulmonary vasodilation also causes an increase in blood oxygen delivery by decreasing the load on the right ventricle, so it is able to pump out more blood (Hsu et al., 2006). In addition, the reduction of pulmonary vasoconstriction would increase the venous return to the left ventricle so that stroke volume and cardiac output would increase and result in a larger circulation of blood to the working tissue (Hsu et al., 2006). Two studies measured stroke volume and concluded that there were significant increases in the volume of blood ejected with Sildenafil compared to the placebo (Ghofrani et al., 2004; Hsu et al., 2006). This supports the idea that pulmonary vasodilation is effective in increasing blood oxygen delivery by decreasing the pressure (afterload: the pressure or force the heart has to contract against to eject blood) on the right ventricle and increasing the venous return at the left ventricle so more blood is pumped by the heart. Ghofrani et al. (2004) recorded an increase in stroke volume in both acute hypoxic and chronic hypoxic environments. This supports that vasodilation is effective in increasing the oxygen available to the body via increasing blood oxygen transport in both lengths of exposure. Stroke volume would be a good factor to measure since it is a valid indicator of pulmonary

vasodilation that is not confounded by the length of exposure; however, due to the lack of funding and access to the appropriate instruments, stroke volume was not measured in this experiment. Oxygen saturation was the only available measure that could be used to suggest occurrence of pulmonary vasodilation in the current experiment. This validates the requirement of the acute exposure set up even more.

Overall, while stroke volume increased, there were some differences in cardiovascular variables between the studies. Slightly different from Ghofrani et al. and Hsu et al., who recorded higher cardiac outputs with no significant change in heart rate, Richalet et al. (2005) concluded that cardiac output was similar with the use of Sildenafil to that without supplementation while heart rate actually decreased with the use of Sildenafil. This suggests that stroke volume increased and was the determining factor for the maintenance of cardiac output (Richalet et al., 2005). Moreover, Richalet et al. did use a larger dose and a chronic dosing pattern which could have resulted in a larger activation of this cGMP pathway. At lower doses, Ricart et al. (2005), Ghofrani et al. (2004), and Hsu et al. (2006) found no significant differences between the heart rates with the different treatments in hypoxia. Furthermore, Ricart et al. (2005) showed a tendency for the ejection fraction (a measure of stroke volume as a percentage of blood volume in the heart) to increase with Sildenafil. This may have been significant if more subjects were used; they had to exclude three of the fourteen subjects. In addition, the results may have been limited due to the time of measurement (after exercise was complete instead of measuring during the last portion of exercise as in the other experiments). Overall, these measurements could have varied due to the use of different instruments. Ricart et al. (2005) and Richalet et al. (2005) both used echocardiography, which is hard to use during exercise and requires a trained and

experienced operator to achieve accurate results. In comparison, Ghofrani et al. (2004) used the gold standard gas-rebreathing technique, while Hsu et al. (2006) used Physioflow impedance cardiography with a device that is not yet validated for the situations of constant work rate exercise, simulated altitude, or Sildenafil supplementation. Despite these differences, these experiments reported an increase in the amount of blood pumped displayed as an increase in stroke volume and cardiac output in addition to a decrease in pulmonary arterial pressure (Ghofrani et al., 2004; Ricart et al., 2005; Richalet et al., 2005). This increase in blood oxygen delivery supports that these pressure results were indicative of vasodilation. Overall, these studies supported that hypoxic pulmonary vasoconstriction is attenuated with pulmonary vasodilation displayed as an increase in oxygen uptake and blood oxygen delivery.

**Effects on performance.** Ghofrani et al. (2004), Richalet et al. (2005), and Hsu et al. (2006) concluded that Sildenafil ultimately improved exercise performance in hypoxia regardless of the type of test and whether work capacity was measured as peak watts,  $\text{VO}_2$  max, or time trial performance. As seen in the measured variables stated earlier, the variability in the improvement was correlated with the type of test, test intensity, degree of altitude, and length of exposure (Hahn & Gore, 2001). When comparing acute hypoxic exposure to chronic exposure, Sildenafil seemed to improve performance during incremental exercise to exhaustion by a greater percentage of power output (peak watts) in the acute environment than in the chronic environment (Ghofrani et al., 2004). This could be a result of several physiological changes seen in chronic exposure and not in acute exposure. An increase in bicarbonate excretion decreases the body's acid-base buffering capacity which

can severely limit intense performance (known to produce a highly acidic environment) for several days at or above altitudes of 2000 m (Bartsch & Saltin, 2008). Furthermore, this increased ventilation may cause dehydration due to exacerbated evaporative loss from humidifying the dryer air characteristic of high altitude at a higher rate for a longer period of time (Sheel et al., 2010), also limiting performance. In addition, alterations in skeletal muscle substrate usage and metabolism could also affect performance (Hahn & Gore, 2001; Martin et al., 2010). Chronic exposure impaired the subjects to a greater extent because the chronic exposure resulted in more negative confounding factors that limit performance indirectly from the chronic reduction of oxygen availability. During chronic exposure, the improvement in exercise performance with Sildenafil supplementation compared to the same exercise test with a placebo was less than that seen during acute exposure. In addition, none of the performances were able to match those performances at normoxia (Gotshall, 2007), which suggests multiple factors of impairment.

When examining intensity of the exercise bout over similar exposure lengths, intensity seemed to influence the degree of improvement of one's exercise performance with supplementation. Ghofrani et al. (2004) used an incremental cycling to maximum test while Hsu et al. (2006) used a lower intensity constant work rate cycling test. The results of the exercise tests showed that the higher intensity maximum test had a higher percentage of improvement with supplementation than the lower intensity constant work rate test. This supports previous knowledge: as intensity increases, the aerobic system is impaired more (Hahn & Gore, 2001), and performance is more impaired. As a result, the physiological changes due to Sildenafil will have a larger impact on the performance. (Ricart et al. (2005) did not measure performance; they only used a constant work rate exercise bout to measure

the physiological variables discussed previously.) Lastly, Ghofrani et al. (2004) and Richalet et al. (2005) have concluded that Sildenafil did not have significant effects on the systemic physiological measures even though there were slight trends of inconsistencies. The systemic circulation is overwhelmed by the hypoxia-induced vasodilation, so that the vasodilatory effect of Sildenafil on the systemic vasculature is not significant. This shows that Sildenafil affects the pulmonary system (Ghofrani et al., 2004; Gotshall, 2007; Richalet et al., 2005) without impairing other physiological adaptations and systems that support exercise (Richalet et al., 2005). Overall, Sildenafil was able to reduce hypoxic pulmonary vasoconstriction by causing vasodilation of the pulmonary system. This pulmonary vasodilation increased both gas exchange and blood oxygen delivery and ultimately improved hypoxic aerobic performance.

**Inter-subject variability.** Besides variability due to different experimental setups and different instruments used, when examining all the physiological measures, Richalet et al. (2005), Ricart et al. (2005), Ghofrani et al. (2004), and Hsu et al. (2006) discovered trends in the variability of responses between the subjects. Variability in subject populations could have had some influence on the variance of the results in these four studies. While Ricart et al. (2005), Richalet et al. (2005), and Hsu et al. (2006) all examined healthy males, Richalet et al. and Hsu et al. more specifically examined moderately trained males and trained male cyclists and triathletes, respectively. Furthermore, Ghofrani et al. (2004) examined healthy males and healthy females who were experienced in traveling to altitudes of 3500 m or 6000 m. Four main characteristics exist along a continuum and have different degrees of influence on one's response to hypoxic exercise and supplementation. First, variability could have

been present due to different levels of previous experience in both the length and the height of high altitude exposure between the subjects. Ghofrani et al. (2004) examined subjects that had previous experience at two different levels of exposure; therefore, the two groups might have responded differently to the same level of high altitude exercise stress due to their acclimatization status (length of exposure and level of altitude) (Martin et al., 2010). Bartsch and Saltin (2008) concluded that those with previous high altitude experience seemed to have a higher threshold before developing symptoms of high altitude illness such as headaches, gastrointestinal upset, fatigue, weakness, dizziness, difficulty sleeping, lack of appetite, and change in mental status than those with no previous experience (Scherrer et al., 1996). This would result in variability and error within the same study and when comparing one study with those of experience, such as the Ghofrani et al. (2004) study, to those with no experience, such as the Richalet et al. (2005) study, if all the other variables were also the same. Secondly, fitness level influences one's response to hypoxic exercise. In addition, Hsu et al. (2006) was able to determine that subjects who were more fit had a greater degree of impairment than those less fit and may have seen more benefit with supplementation. Not only are training status and acclimatization status factors in determining one's response to high altitude exercise, but genetics also plays a role (Martin et al., 2010). Hsu et al. (2006) discussed variability in the degree of the response to the high altitude stress and also the response with Sildenafil. They, in addition to other researchers, concluded that those who were more impaired by high altitude (prone) also seemed to show the most improvement with Sildenafil supplementation (responder) (Gotshall, 2007; Hsu et al., 2006). Thirdly, inter-subject genetic variability seems to determine the degree of response to the type of supplement (responder vs. non-responder) and the degree of whether they are prone or

resistant to hypoxic vasoconstriction expressed along a continuum of symptoms. Finally, sex may have some influence on one's response to the type of supplementation, but Ghofrani et al. did not evaluate if there were differences in responses between males and females, which could have also led to some of the variability.

To control for adaptations and this variability, this experiment only used male athletes of sufficient training status. Both previous and current training experience was considered via examining training reports and fitness testing. The level of previous altitude exposure was also delimited in the participant inclusion criteria to control for physiological adaptations. Overall, even though there was variability, these studies provided evidence to support that pulmonary vasodilation reduces hypoxic vasoconstriction and improves hypoxic performance.

### **Three Sources of Nitric Oxide Cause Vasodilation**

**Endogenous.** The body does have a system that naturally responds to the stimulus of hypoxia with a mechanism of vasodilation in the systemic circulation (Blitzer et al., 1996). Nitric oxide production is regulated by endothelial cells which are responsible for controlling vascular tone (Blitzer et al., 1996). Low oxygen availability stimulates endothelial cell enzymes to induce systemic vasodilation as a protective mechanism of increasing blood supply and therefore oxygen to meet the metabolic demands of systemic organs and working tissue (Blitzer et al., 1996). Previous research has shown that the body naturally converts L-arginine into nitric oxide which activates a cGMP linked chain reaction to induce vasodilation (Zuckerbraun et al., 2011). This is the same pathway that the PDE-5 inhibitors manipulate to cause pulmonary vasodilation (Zuckerbraun et al., 2011). In contrast, the



pulmonary circulation in humans undergoes a hypoxic reflex of pulmonary vasoconstriction (Bailey et al., 2009). Low oxygen availability elicits a protective response of constricting pulmonary blood vessels so that perfusion to poorly oxygenated areas is minimized to help improve oxygen transfer through matching ventilation of the lung to perfusion of the pulmonary capillaries (Blitzer et al., 1996). Blitzer et al. (1996) examined the role of endogenous nitric oxide production and the degree of vascular response to an acute hypoxic stimulus in relation to both the systemic and pulmonary systems via measurements of systemic and pulmonary blood pressures in healthy men and women. First, Blitzer et al. (1996) showed that the nitric oxide synthase enzyme was responsible for the release of nitric oxide and thus regulating vascular tone since both pulmonary and systemic vascular pressures increased at normoxia with infusion of a nitric oxide synthase inhibitor (L-NMMA). Then, during acute exposure to hypoxic gas, systemic vascular resistance decreased while pulmonary vascular resistance increased (Blitzer et al., 1996). Finally, when hypoxic exposure was maintained while L-NMMA was infused, systemic vascular resistance increased, and pulmonary vascular resistance increased even more compared to the hypoxia alone condition (Blitzer et al., 1996). This shows that the same mechanism is responsible for vasodilation in both systemic and pulmonary circulations; however, it seems to be impaired in the pulmonary system during hypoxia. As a result, supplementation has been an idea of interest to assist nitric oxide levels with vasodilation in the pulmonary system.

**Exogenous: nitrite or nitric oxide.** Supplementation via nitrite and nitric oxide has been examined in relation to the pulmonary system. Nitrite has previously been shown to be reduced to nitric oxide ultimately to initiate vasodilation (Zuckerbraun et al., 2011).

Zuckerbraun et al. (2010) determined that nebulized nitrite was effectively able to reduce hypoxia-induced pulmonary hypertension in sedated rats and mice without affecting systemic arterial pressure. With the use of inhibitors in both in vivo sedated animals and in vitro cell cultures, nitrite was determined to induce vasodilation via producing nitric oxide which increases cGMP activity (Zuckerbraun et al., 2010). Furthermore, Zuckerbraun et al. found that this mechanism was dependent on tissue specific nitric oxide stimulating enzymes which are found in the pulmonary vasculature (Zuckerbraun et al., 2010). Along with the level of tissue-specific enzyme activity, the level of activation of this mechanism has also previously been shown to be dependent on pH and oxygen concentration of the environment (Zuckerbraun et al., 2010). When examining in vitro rat aortas, it was concluded that nitric oxide was generated from physiological level doses of nitrite (via sodium nitrite) to induce vasodilation in a pH dependent fashion (Modin et al., 2001). The degree of vasodilatory activity was correlated with nitric oxide and nitrite concentrations (Modin et al., 2001). Vasodilation was increased in more acidic environments paralleling those seen in tissues during “hypoxia and increased metabolic activity” (Modin et al., 2001, p.15). This suggests that the application of nitric oxide could improve pulmonary blood flow in hypoxic environments.

While much of the research in examining the pulmonary vasodilatory effects of exogenous sources of nitric oxide have been examined in animals due to the invasiveness of pulmonary vascular measures, relatively little research in this area has been done using human subjects. Those that did examine human subjects did so via estimates with indirect measures of pulmonary arterial pressure. Ingram et al. (2010) analyzed the pulmonary circulation in response to infusion of low doses of sodium nitrite (to result in a plasma nitrite

level not more than double that of physiological levels) in healthy male humans at rest by estimating pulmonary vascular resistance with pulmonary arterial pressure measured via transthoracic echocardiography. In addition, they examined systemic vascular resistance differences measured via strain gauge plethysmography of the forearm (Ingram et al., 2010). They concluded that both systems showed evidence of vasodilation after nitrite infusion in a 12% oxygen hypoxic environment (Ingram et al., 2010) (similar to the environment used in this study). The pulmonary system in that study showed prolonged effects of vasodilation after plasma nitrite levels returned to baseline while these effects ceased in the systemic system (systemic effects were correlated with plasma nitrite levels) (Ingram et al., 2010). Even though echocardiography is an indirect measure and can be affected by variables such as regurgitation, Ingram et al. (2010) concluded that these results were reliable since they were consistent with the results that used pulmonary acceleration time and isovolumic relaxation time to help assess pulmonary arterial pressure. They suggested that pulmonary tissue may have a lower threshold or higher degree of sensitivity to exogenous sources of nitric oxide since it constricts in response to hypoxic environments while the systemic vasculature already dilates in response to hypoxic environments (Ingram et al., 2010). This supports that exogenous nitrite can cause pulmonary vasodilation in healthy males at rest.

Likewise, the effect of inhalation of nitric oxide has been examined in relation to pulmonary vasoconstriction resulting from acute hypoxic exposure (10%  $F_{I}O_2$ ) and chronic hypoxic exposure (4559 m of altitude) in men and women who had a history of being prone to high altitude pulmonary edema and hypertension with those whom were resistant as determined from previous high altitude exposure (Scherrer et al., 1996). Similar to the PDE-5 inhibitor studies, nitric oxide reduced pulmonary arterial pressure, measured indirectly via

echocardiography, in both groups but more substantially in those prone to edema and hypertension (Scherrer et al., 1996). This could be due to the responders (prone) and non-responders (resistant) situation described earlier; those impaired the most are more likely to have larger benefits (improvements) with supplementation. When examining the oxygen saturation of those under chronic hypoxic exposure after a several day sojourn, nitric oxide inhalation improved oxygen saturation in the prone subjects that did experience symptoms of edema (Scherrer et al., 1996). Nitric oxide inhalation did not impair subjects that were prone but did not have current symptoms of edema based on radiography (Scherrer et al., 1996). Finally, application of nitric oxide reduced oxygen saturation in those resistant to edema (Scherrer et al., 1996). This suggests that the nitric oxide helped the ventilation-perfusion matching in the first two groups of subjects while the resistant subjects did not have significant mismatches (Scherrer et al., 1996). The third group of subjects were impaired because the added nitric oxide had to displace some of the partial pressure of oxygen (-4%) in the inhaled gas resulting in a lower oxygen content in the treatment gas (Scherrer et al., 1996). This is supported by the experiment in acute exposure (minutes) where both prone and resistant groups did not see improvement in their blood oxygen saturation (Scherrer et al., 1996). The additional reduction in oxygen affected them similarly since they had not had time to adapt and improve ventilation-perfusion matching (chronic resistant) or develop edema that would further prevent ventilation-perfusion matching and gas exchange (chronic prone). Furthermore, radiography was used to examine lung-perfusion in the subjects during the chronic altitude exposure (Scherrer et al., 1996). Scherrer et al. (1996) was able to conclude that inhalation of nitric oxide effectively redistributed blood flow away from regions of poor ventilation and high edema to those regions of better ventilation and low

edema, improving gas exchange and oxygen saturation in those with pulmonary edema. This suggested that nitric oxide reduces the effects of pulmonary vasoconstriction via decreasing pulmonary arterial pressure and rerouting blood flow to better ventilated areas to improve gas exchange in men and women at rest during hypoxic exposure. A major disadvantage of these types of supplementation is that the mechanisms of inhalation and infusion are not practical in a field environment; therefore, supplementation via dietary nitrate ingestion may be a more applicable method.

**Exogenous: dietary nitrate.** Supplementation via dietary nitrate ingestion has been examined to determine its vasodilatory potential. Dietary nitrate is converted into nitrite by bacteria produced enzymes in saliva and the oral cavity (Zuckerbraun et al., 2011). Nitrite then reacts with tissue specific enzymes to produce nitric oxide (Zuckerbraun et al., 2011). Due to the invasiveness of pulmonary vascular resistance measures, much of the research is based on animal models or with the use of indirect measures to estimate pulmonary vascular resistance in humans; therefore, most of the studies on dietary nitrate supplementation with humans only examine the vasodilatory effects on systemic vasculature and not pulmonary vasculature. Studies have shown that dietary nitrates do have vasodilatory and blood pressure reduction properties when examining systemic vasculature in both humans (Webb et al., 2008) and animals (Kapil et al., 2010).

Kapil et al. (2010) compared blood pressure reduction and flow mediated dilation of the brachial artery in response to potassium nitrate, potassium chloride, and beetroot juice in healthy men and women during rest at normoxia. They found that potassium nitrate of three different doses (248 mg, 744 mg, and 1488 mg of nitrate) significantly decreased systemic

blood pressure compared to potassium chloride and that the blood pressure effects followed a nitrate ingested dose dependent relationship with plasma nitrite and plasma cGMP concentrations measured using ozone chemiluminescence (Kapil et al., 2010). This supports nitrate as an initiator of these changes via activation of the same second messenger (cGMP) as PDE-5 inhibitors that have been shown to cause pulmonary vasodilation. Likewise, 250 mL of (5.5 mMol nitrate) beetroot juice also caused a significant decrease in systemic blood pressure that paralleled plasma nitrite and cGMP concentrations compared to 250 mL of water (Kapil et al., 2010). These results showed that a nitrate containing juice has blood pressure lowering effects.

Kapil et al. utilized infrared irritation to mediate vasodilation in the brachial artery. When examining flow mediated dilation of the brachial artery after an IR irritation, both beetroot juice and potassium nitrate significantly increased the diameter of the artery from before the IR stress (Kapil et al., 2010). This supported that nitrates have vasodilatory effects in response to stress independent of the type of source (natural or pharmacological). These statistically significant blood pressure reductions and systemic vasodilation results were also supported by a similar study with a larger nitrate dose of 500 mL ( $45.0 \pm 2.6$  mMol/L nitrate (22.5 mMol of nitrate)) of beetroot juice (Webb et al., 2008). Furthermore, both studies reported that these changes were correlated with the peak plasma nitrite concentration at 2.5-3 hours after nitrate ingestion (Kapil et al., 2010; Webb et al., 2008).

Additionally, Kapil et al. (2010) found a sex specific response to nitrate supplementation by revealing sex specific differences in blood pressure reduction and plasma nitrite concentration after body weight was normalized in post hoc analysis. They suggested that these differences were due to sex differences in nitrate processing: premenopausal

females had a higher plasma nitrite concentration after ingestion while they experienced a smaller reduction in blood pressure (Kapil et al., 2010). This has been supported previously when examining nitric oxide synthase expression and activity comparing age-matched females and males (Kapil et al., 2010). Increased basal sensitivity could be the reason why females have lower resting blood pressures than males without supplementation, which could become saturated with the addition of nitrate via supplementation and result in less of a reduction in blood pressure (Kapil et al., 2010). Kapil et al. (2010) did not control for the influence of hormone variation during the menstrual cycle. Since sex and hormones may further confound results, this experiment controlled for these influences by only examining male subjects. Overall, these experiments related the dietary nitrate-induced reduction of systemic blood pressure to systemic vasodilation.

***Beetroot juice.*** Red beetroot juice is a commonly used source for dietary nitrate supplementation. Red beetroot is a vegetable with a very high nitrate content (classified as greater than 250 mg/100 g fresh weight) compared to other natural sources of nitrate (Hord et al., 2009). In addition, it has been shown that nitrate rich vegetables, such as red beetroot, have approximately a 100% bioavailability of nitrate absorption from oral supplementation whether cooked or consumed raw in healthy subjects aged 18-35 years old (van Velzen, Sips, Schothorst, Lambers, & Meulenbelt, 2008). Beetroot is commonly administered as juice, and the nitrate content of juice has been shown to be well preserved by refrigeration for twenty-four hours after opening (Tamme et al., 2010). Even though beetroot juice may have a slower activation time, it has a more practical oral administration compared to inhalation or infusion of nitric oxide or nitrite, respectively (Zuckerbraun et al., 2011). Additionally, this

natural source of nitrate appears to have fewer side effects (red urine and stools) (Bailey et al., 2009; Gilchrist et al., 2010; Vanhatalo et al., 2010; Webb et al., 2008) when compared to other supplements such as PDE-5 inhibitors (headaches, dizziness, flushing, dyspepsia) (Gotshall, 2007; Hsu et al., 2006) at this acute dose. For these reasons, beetroot juice is a commonly used source of exogenous nitrate supplementation in humans and was used as the nitrate source in this study.

Overall, sources of exogenous nitrite oxide have been shown to cause pulmonary vasodilation; however, dietary nitrates have only been examined to cause systemic vasodilation in humans due to the invasiveness of direct measures and cost of indirect measures. It is unknown if dietary nitrates, such as red beetroot juice, cause vasodilation in the pulmonary system. This study was designed to monitor indirect indicators of pulmonary vasculature tone during beetroot juice supplementation under hypoxic conditions.

### **Nitrate Effects on Performance**

Endogenous nitric oxide has been shown to cause vasodilation to help improve blood flow and delivery in systemic vasculature to support the oxygen demands of working tissue (Blitzer et al., 1996). Furthermore, under acidic and hypoxic environments, such as those created during exercise and high altitude, respectively, tissue specific enzyme production of nitric oxide can be enhanced along with the activity of enzymes that convert nitrite to produce nitric oxide (Rassaf et al., 2007). While nitrite is converted to nitric oxide under hypoxic and acidic conditions, nitric oxide in the plasma is oxidized back to nitrite in normal resting conditions (Rassaf et al., 2007). Rassaf et al. (2007) concluded that plasma nitrite is an oxidation product of nitric oxide production and represents the capacity of the body's



ability to produce nitric oxide. They examined plasma nitrite levels and flow-mediated dilation of the brachial artery in healthy males and females during a stepwise increase to maximum exercise test (Rassaf et al., 2007). Rassaf et al. (2007) determined that exercise intensity was positively correlated to the post exercise plasma nitrite levels which paralleled vasodilation (level of mediated blood flow). Furthermore, these post exercise plasma nitrite levels were positively correlated with the maximum exercise power achieved but were negatively correlated with age (Rassaf et al., 2007). Administration of a nitric oxide synthase inhibitor after exercise inhibited plasma nitrite production, supporting that nitric oxide was produced by this mechanism in response to exercise (Rassaf et al., 2007). Rassaf et al. were able to conclude that post exercise plasma nitrite levels: “the capacity of the vasculature to produce nitric oxide...together with age independently predicts the maximal stress achieved and duration of exercise.” (Rassaf et al., 2007, p.672) These results linked exercise performance with the ability to produce nitric oxide. Because age is a confounding factor of performance and nitric oxide production, this study only recruited subjects from a specific age range: 18-30 years old, due to access and the applicability of the competitive opportunity associated with this age. In conclusion, increased nitrite and therefore nitric oxide production is related to increased exercise intensity and performance.

**Beetroot juice effects on performance.** The nitrates in red beetroot juice have been shown to increase aerobic performance at normoxia (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010). Similar to the dietary nitrate vasodilatory and blood pressure reduction studies, these beetroot juice studies have also only examined the physiological effects due to nitrate supplementation in the systemic circulation suggesting only systemic

methods of exercise improvement via beetroot juice. Vanhatalo et al. (2010) utilized a double blind crossover study to compare acute (one dose 2.5 hours before test) and chronic (5 and 15 days) daily supplementation of 0.5 L of beetroot juice (5.2 mMol of Nitrate) to 0.5 L of cordial juice placebo supplement in both healthy males and females. In this investigation, cyclists performed two five minute moderate intensity (90% GET-gas exchange threshold ~ anaerobic threshold) submaximal constant work rate exercise tests and a ramp incremental to exhaustion exercise test with each test separated by ten minutes (Vanhatalo et al., 2010). Vanhatalo et al., reported a lower oxygen cost in the submaximal test, 2.5 hours after the first supplementation, that was maintained throughout the fifteen days (Vanhatalo et al., 2010). Vanhatalo et al. (2010) reported that there were no significant changes in heart rate during exercise, which Kapil et al. (2010) reported at rest, suggesting that nitrates do not affect the contraction rate of the heart when affecting cardiac output and blood delivered. When examining the incremental step to exhaustion test, the work rate associated with GET and peak power output did not significantly increase from the placebo and baseline until the test administered after fifteen days of supplementation (Vanhatalo et al., 2010). In a similar study, Bailey et al. (2009) utilized a double blind crossover study to examine the last three days of (a six day) chronic supplementation of beetroot juice (5.5 mMol of nitrate/day) or blackcurrant cordial juice in recreationally active men. In this investigation, cyclists performed different tests across the last three days of supplementation. They performed two six minute moderate intensity (80% GET) submaximal constant work rate cycling tests on day 4. During the last two days, they performed one six minute moderate constant work rate test and one severe intensity (70% difference between 80% GET and  $\text{VO}_2$  peak) constant work rate test either 6 minutes long on day 5 or until exhaustion on

day 6 with each test separated by twenty-five minutes (Bailey et al., 2009). Bailey et al. (2009) observed similar results to Vanhatalo et al. (2010) finding a reduction in oxygen cost during the submaximal cycling test. Furthermore, both Vanhatalo et al. and Bailey et al. reported a significantly elevated plasma nitrite level that was constant throughout supplementation and a reduced systemic blood pressure (Bailey et al., 2009; Vanhatalo et al., 2010), showing no evidence of reduced sensitivity to chronic supplementation of nitrates (Vanhatalo et al., 2010). Additionally, Bailey et al. (2009) discovered significantly increased blood flow and reduced muscle fractional oxygen extraction (supply : utilization) with beetroot supplementation during the moderate intensity test suggesting that muscle energy production became more efficient since oxygen consumption decreased during the moderate constant work rate bout. The results of Bailey et al. (2009) also showed that time to exhaustion (exercise tolerance) was increased in the severe intensity constant work rate test with chronic supplementation of beetroot juice compared to placebo; however, this was done with a change in oxygen kinetics (the oxygen uptake slow component was reduced) without an alteration in fractional oxygen extraction. The continued increase in oxygen consumption seen with heavy to severe intensity constant work rate exercise (Bailey et al., 2009) was reduced so that it took longer to reach maximum oxygen consumption, extending performance. Overall, these results showed that supplementation has both acute and chronic effects improving performance.

The acute effects of improvement may be due to increased blood and oxygen delivery to the working muscles (Blitzer et al., 1996). The chronic effects were suggested to be due to peripheral adaptations. Vanhatalo et al. (2010) suggested adaptations (along with Bailey et al. (2009)) in the skeletal muscle due to the exogenous nitric oxide exposure such as reduced

muscle ATP turnover for the same external work rate, which would reduce phosphocreatine degradation and accumulation of adenosine diphosphate and inorganic phosphate shown in a previous study by this group. They hypothesized that nitrates could have resulted in more efficient maintenance of sarcoplasmic calcium homeostasis and or myosin ATPase activity which would reduce the stimulation of oxidative phosphorylation and muscle fatigue progression (Vanhatalo et al., 2010). Vanhatalo et al. (2010) also suggested chronic supplementation could have resulted in improvements via changes in muscle oxygen delivery and motor unit recruitment. In addition to increased oxidative metabolic enzyme activity (Bailey et al., 2009), Vanhatalo et al. (2010) also concluded that chronic exposure could be linked to increased mitochondrial mass based off a previous study which supported that mammalian cells increased mitochondrial biogenesis via cGMP pathways when exposed to nitric oxide for six days. These experiments were limited in their conclusions because beetroot juice is also high in polyphenols which have previously been linked to a protein responsible for mitochondria biogenesis and to increased exercise tolerance (Vanhatalo et al., 2010). Consequently, this study used a nitrate depleted beetroot juice placebo to compare with the nitrate rich beetroot juice to examine the nitrate dependent effects on oxygen availability, vascular activity, and performance. A control group with just water was also used to control for the placebo effect and give comparison of the effects of non-nitrate compounds on these variables. Nonetheless, these experiments still supported that beetroot juice improves exercise performance by reducing oxygen cost during submaximal intensity exercise (Bailey et al., 2009; Vanhatalo et al., 2010), improving high intensity exercise tolerance (Bailey et al., 2009; Vanhatalo et al., 2010), and improving the work rate associated

with GET and peak power output during a ramp incremental test to exhaustion (Vanhatalo et al., 2010).

While tests to exhaustion and constant work rate tests showed valid improvements in performance, they are not as applicable to competitive situations as simulated time trials. Athletic performance is usually based on time of completion in which the work rate is self-selected. Time trials have been shown to be more reliable than constant work rates tests to exhaustion (Jeukendrup, Saris, Brouns, & Kester, 1996). Jeukendrup et al. (1996) analyzed the performances of five repeated trials of a time trial protocol compared to a continuous exercise (at 75% maximal power output) until exhaustion protocol of a similar length (approximately one hour) on an electronically braked cycle ergometer. They observed individual coefficients of variance for each subject ranging from 0.8% to 5.8% for the time trial and 17.4% to 39.5% for the continuous exercise to exhaustion protocol, respectively (Jeukendrup et al., 1996). In addition, Palmer et al. (1996) found similar variances of performance times between the completion of three trials of a 20 km and three trials of a 40 km time trial (coefficient of variance:  $1.1 \pm 0.9\%$  and  $1.0 \pm 0.5\%$ , respectively) on an air-braked cycle ergometer suggesting that time trials are reliable, reproducible tests. Furthermore, significant correlations were observed between time trial performance times and performance times during actual road races ( $r = 0.98$ ) when the 40 km laboratory test was compared to a 40 km road race (Palmer, Dennis, Noakes, & Hawley, 1996). Therefore, not only are time trials a reliable measure, but they also may be good predictors and result in effective evaluations of field performance. A time trial of 200 kJ on a isokinetic cycle ergometer, one of similar length to that used in this study, has been shown to result in times with a low range of variation (coefficient of variation:  $\pm 0.95$ ) (Hickey, Costill, McConnell,

Widrick, & Tanaka, 1992). Low levels of variation would lead to a more reliable assessment to determine if the treatment is effective in improving performance. More specifically, athletes would be interested in whether the acute supplementation of beetroot juice can help improve race performance, thus improving their time and making them more competitive. Acute supplementation should be analyzed during a time trial performance test to determine this.

***Time trial performance.*** One double blind crossover study examined the effects of acute ingestion of 0.5 L of beetroot juice (6.2 mMol of nitrate) compared to 0.5 L of nitrate depleted beetroot juice (~0.0047 mMol of nitrate) in healthy male competitive club cyclists (mean age  $21 \pm 4$  years; similar to subjects in this experiment) on 4 km and 16.1 km time trial performance (Lansley et al., 2011). While oxygen consumption ( $\text{VO}_2$ ) did not significantly differ between the treatment groups, power output (PO) significantly increased, systolic blood pressure decreased, and both 4 km and 16.1 km race performances improved by a similar amount with the beetroot juice compared to the placebo (Lansley et al., 2011). These results suggest that it was the nitrates in beetroot juice (supported by elevated plasma nitrite levels) that were responsible for improving performance and cycling economy (as displayed by higher PO for the same  $\text{VO}_2$ ) in events of distances between 4 and 16.1 km (Lansley et al., 2011). In addition, these results showed that only an acute dose was needed to improve performance even though this dose of 6.2 mMol of nitrate was 4-12 times greater than 40-100 mg/day (Mensinga, Speijers, & Meulenbelt, 2003), the typical daily dietary intake in the United States (Lansley et al, 2011). Lansley et al. (2011) did recognize a few limitations of their study, such as that some compounds that could be responsible for these physiological

changes may require nitrate to become activated, in addition to the lack of monitoring the diet, hydration status, and quality of sleep of the subjects. They also did not control for the placebo effect or the influence of other non-nitrate beetroot compounds. In addition to using a control containing water, this study also required a twenty-four hour pre-supplemental trial log to monitor diet, hydration, and sleep to help draw a more accurate conclusion. Besides these limitations, these results strongly supported that the nitrates in beetroot juice improve time trial performance at normoxia. It is still unknown if dietary nitrates can help improve aerobic performance at high altitude or hypoxia.

Cyclists commonly have to compete in races at moderate to high altitude (Hahn & Gore, 2001; Martin et al., 2010). Because these aerobic performances at high altitude are so impaired by hypoxia (Hahn & Gore, 2001; Martin et al., 2010), dietary nitrate oral supplementation may be a potential natural and practical mechanism to reduce the impairment, increasing performance and making athletes more competitive at high altitude. Like the previous example, this study used trained club level cyclists and triathletes during acute hypoxic exposure with a similar amount of acute nitrate supplementation to simulate closely a real situation in which the competitive athlete just ascends for the race. The recruited subjects likewise had a significant previous experience of cycling and completing these races to rule out familiarization bias. A race of intermediate length (approximately 200 kJ) was used to tax the aerobic system that is so highly relied on for race performance and impaired by high altitude/hypoxic exposure (Hahn & Gore, 2001). Size of the subject can often affect results in the field due to factors such as wind resistance (Hahn & Gore, 2001; Smith, Davison, Balmer, & Bird, 2001); therefore, energy expenditure was used to factor out this influence and make it more applicable to simulated competition.

Similar to the Lansley et al. (2011) study, oxygen consumption and race time were measured along with heart rate and systemic blood pressure to analyze potential improvements, but the ability to measure plasma nitrite concentration via chemiluminescence technique was not accessible or feasible for this experiment. Based on previous research that has shown that hypoxic and acidic environments enhance nitric oxide production (Modin et al., 2001), the amount of nitrates in red beetroot juice that have been shown to be effective during exercise at normoxia should also be effective during exercise at hypoxia, a more hypoxic and acidic environment than exercise at normoxia. A similar amount of nitrate content via the same brand of beetroot juice used in the Lansley et al. study at normoxia was used in this experiment to examine its effects on performance at hypoxia. In conclusion, there is evidence that red beetroot juice helps improve aerobic exercise performance at normoxia; however, it is still unknown if red beetroot juice improves aerobic exercise performance at hypoxia.

## **Conclusion**

Overall, previous research has shown that supplemental pulmonary vasodilators, such as PDE-5 inhibitors, are capable of increasing arterial blood oxygen saturation and blood oxygen delivery that are reduced as a result of hypoxic pulmonary vasoconstriction (Ghofrani et al., 2004; Hsu et al., 2006; Richalet et al., 2005). These PDE-5 inhibitors have also been shown to improve aerobic exercise performance at high altitude and hypoxia (Ghofrani et al., 2004; Hsu et al., 2006; Richalet et al., 2005). While exogenous nitrite and nitric oxide have been reported to cause pulmonary vasodilation (Ingram et al., 2010; Scherrer et al., 1996; Zuckerbraun et al., 2011), dietary nitrates have only been tested and shown to cause



vasodilation in the systemic circulation (Kapil et al., 2010; Webb et al., 2008). Dietary nitrates, such as red beetroot juice, have also been shown to increase athletic aerobic exercise performance (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010), but this has only been tested at normoxia. It is unknown if dietary nitrates can decrease arterial blood oxygen desaturation that results from hypoxia-induced pulmonary vasoconstriction and lead to an increase in aerobic exercise performance at hypoxia or high altitude. The purpose of this study was to determine the effect of short-term red beetroot juice supplementation on systemic blood pressure, exercising arterial blood oxygen saturation, and acute aerobic (approximately 200 kJ) time trial performance during acute hypoxic exposure (simulated high altitude (4300 m)) in male club level cyclists and triathletes.

## **Experiment**

### **Methodology**

It is unknown if dietary nitrates, such as red beetroot juice, can decrease the arterial blood oxygen desaturation that results from hypoxic pulmonary vasoconstriction and lead to an increase in aerobic exercise performance at hypoxia or high altitude. The purpose of this study was to determine the effect of short-term red beetroot juice supplementation on systemic blood pressure, exercising arterial blood oxygen saturation, and acute aerobic (approximately 200 kJ) time trial performance during acute hypoxic exposure (simulated high altitude (4300 m)) in male club level cyclist and triathletes.

**Subjects.** Nine healthy trained competitive male cyclists and triathletes (18-30 years old) were recruited from the Appalachian State University Cycling team and local cycling community to insure consistent time trial performances and limit the familiarization effect of cycling throughout the experiment. All athletes had at least one year of competitive experience and had been currently undergoing regular training sessions of at least three days per week for the last eight weeks. Additionally, all participants lived at 1300 m (4000 ft) and below for the past six months with occasional traveling excursions above 1300 m (4000 ft). Individuals who made extended sojourns (longer than 2 days) above 4000 feet over this time period were excluded from the study. Individuals were excluded from this study if they had a previous or current history of one or more of the following: smoking, pulmonary hypertension, high altitude pulmonary edema, cardiopulmonary disease, cardiovascular

disease or a current physical condition that impaired physical activity. Females were not allowed to participate in this study due to the confounding factor of possible hormone linked nitrate processing (Kapil et al., 2010). Participants were not allowed to use aspirin, ibuprofen, or compounds containing acetaminophen during the study. No subjects previously used or were currently using dietary supplements in the past month for fat soluble supplements or past week for water soluble supplements.

Before participating in the exercise tests, subjects completed a health history questionnaire and an ACSMAHA Screening Questionnaire along with a training history to examine past training status and current training intensity, volume, and level of competition. Height, weight, and blood pressure of each subject were measured before he began the exercise tests.

All participation was voluntary. After the procedures, risks, and benefits of the study were explained, all subjects gave their written informed consent to participate. This study was approved by the Institutional Review Board of Appalachian State University prior to its implementation.

### **Research design.**

*General study design.* During the first visit, all subjects underwent an initial screening and a pre-supplementation test of initial aerobic cycling fitness (VO<sub>2</sub> maximum test to exhaustion) along with a practice approximately 200 kJ time trial. All subjects then returned on three more occasions to perform one approximately 200 kJ experimental cycling time trial using a different treatment each visit. The treatments were ingested 2.5 hours before the test and contained 70 ml of organic beetroot juice (BR), 70 ml of organic nitrate-

depleted beetroot juice (PL) (both similar to that used in a study by Lansley et al. (2011)), or 70 ml of water (CO). Treatments were administered in a double blind, randomly assigned, crossover design. Subjects and investigators were blinded to the treatments. The subjects were not able to discriminate between the beetroot juice and the placebo due to similar color, taste, texture, and odor (Lansley et al., 2011). Pulmonary, cardiovascular, and metabolic measurements in addition to performance results were examined throughout all three performance trials. Each experimental trial was separated by a washout period of at least forty-eight hours but no more than one week. All practice tests and experimental tests for each subject were done at the same time of day with the same number of days between trials.

***Hypoxic exposure.*** During all of the experimental performance tests, each subject was acutely exposed to hypoxic gas similar to the set up used in a previous study (Morris, Kearney, & Burke, 2000). The hypoxic gas was released from a gravimetrically certified G sized cylinder through a Hans Rudolph mouth piece (7600, V2; Hans Rudolph Inc., Kansas City, MO) containing a two-way non-rebreathing Hans Rudolph valve connected via 3.18 cm I.D. plastic tubing to the gas reservoir (Morris et al., 2000). The reservoir was a 120 L meteorological balloon modified to allow simultaneous filling from the cylinder to allow gas delivery to the subject at ambient barometric pressure (Morris et al., 2000). The breathing gas contained a fraction of inspired oxygen ( $F_{I}O_2$ ) of 12.97-13.02% (partial pressure of oxygen in the inspired gas of about 88.4 Torr). The hypoxic gas was measured before each test to insure oxygen content remained consistent throughout all exercise tests. Subjects wore a nose plug to ensure all exchanged gases were measured.

**Intervention.** Three supplementation treatments were used, and each subject experienced each treatment. The supplemental fluid contained either 70 ml of organic beetroot juice (BR) (containing ~0.4g, 6.45 mMol of nitrate; Beet it Sport Stamina Shot, James White Drinks Ltd., Ipswich, UK), 70 ml of organic nitrate depleted beetroot juice (PL) (containing ~0.048 mMol of nitrate; Beet it Sport Stamina Shot Placebo, James White Drinks Ltd., Ipswich, UK), (similar to that used in a study by Lansley et al. (2011)), or 70 ml of water (CO). The organic beetroot juice was placed through an ion exchange resin selective for nitrate ions to create the nitrate-depleted beetroot juice placebo (Lansley et al., 2011). The PL was otherwise similar to the beetroot juice in color, taste, texture, and odor (Lansley et al., 2011).

Subjects were contacted via phone or email prior to the first pre-supplementation visit and notified of the pre-participation requirements. All subjects were requested to maintain a consistent diet, training program, and physical activity schedule throughout the experiment. In addition, they were instructed to consume similar meals and perform a similar degree of physical activity the day before and the day of the performance test for all three experimental trials. They were provided with a list of foods which they were required to abstain from eating since they are high in nitrate and antioxidant content. Subjects were asked to abstain from caffeine and alcohol twenty-four hours prior to each performance test. They were required to keep a log of all fluids and food consumed along with all physical activity performed and quality and quantity of sleep obtained twenty-four hours before each visit. They were instructed to avoid strenuous physical activity (no more than thirty minutes at equal to or greater than 60% maximum heart rate) twenty-four hours before each performance trial. Participants were told to drink one liter of fluids the night before and one

liter of fluids the morning of each visit. Subjects were required to abstain from consuming any foods or fluids, except water, four hours prior to each visit. Two hours prior to arrival, the subject consumed a standardized 460 kcal meal consisting of complex and simple carbohydrates (two Chocolate Chip Clif Bars, Clif Bar & Company, Emeryville, CA) and one liter of water. Upon arrival, they were not allowed to eat or drink (other than the treatment fluid) three hours before the performance trial. Furthermore, they were not allowed to drink or consume any fluids or food after the treatment was ingested until after the performance trial was complete. Finally, participants were not allowed to use chewing gum or anti-bacterial mouthwash forty-eight hours prior to the performance test since these are known to destroy the oral bacteria necessary for the conversion of nitrate to nitrite (Govoni, Jansson, Weitzberg, & Lundberg, 2008). Subjects were reminded of these requirements at the end of each visit to insure consistent results for the following visits.

### **Experimental procedure.**

***Pre-supplementation period.*** On the same day as the screening, participants performed both a  $\text{VO}_2$  maximum test to exhaustion and a practice time trial under a protocol used in a previous study (Morris, Shafer, Fairbrother, & Woodall, 2011). All tests were performed using an electronically braked cycle ergometer (Lode Excalibur Sport Cycle Ergometer, Groningen, Holland) adjusted to the dimensions of the subject's own bicycle. These pre-supplementation tests were completed under normoxic conditions. For the warm up and  $\text{VO}_2$  max test, the cycle ergometer was placed in a manual mode that allowed the investigator to control manually the timing and application of the workloads. All warm-ups and tests were manually controlled by the same investigator. The warm-up consisted of a

total of ten minutes starting at a power output of  $(3 \times \text{mass (kg)}) - 100 \text{ W}$  for each subject which then increased by 10 W every minute (Morris et al., 2011). Subjects rested for five minutes while equipment was prepared for the graded exercise test. During the  $\text{VO}_2$  max test, each stage was one minute long (Morris et al., 2011). The power output for the first stage was set at:  $W = 3 \times \text{mass (kg)}$ , and each subsequent stage was increased by  $0.3 \text{ W} \times \text{mass (kg)}$  (Morris et al., 2011). Oxygen consumption, respiratory exchange ratio (RER), and heart rate were monitored in fifteen second intervals throughout the test. Maximum oxygen consumption ( $\text{VO}_2$  max) was determined based on these variables. Subjects were allowed to cool down with a comfortable load specified by the subject and to walk around while the equipment was prepared for the practice performance trial.

The practice time trial performance test was performed on the same cycle ergometer fifteen to twenty minutes after the maximum test. The practice time trial was used to allow the subjects to become familiar with manually adjusting the work rate. A warm-up was not necessary before this test. The Lode cycle ergometer was set up to allow manual work rate changes. Subjects were encouraged to complete the trial as fast as they could. The length of the time trial was determined based on the subject's body weight ( $\text{Length} = 2.5 \text{ kJ} / \text{kg of body weight}$ ). The only feedback that the subjects received was the work performed (distance) displayed by the digital readout on the Lode ergometer ( $16.4 \text{ kJ} / 1 \text{ km}$ ); subjects were blinded to the actual work rates, time, power output, or any other performance measures. Subjects were not informed of their results on any test during the study. Subjects received similar verbal encouragement by the test administrators throughout all tests.

***Supplementation period.*** Each subject returned to the Vascular, Biological, and Autonomic Studies Laboratory at Appalachian State University, room 186C, University Hall, on three separate occasions of equal spacing at the same time of day. The first experimental performance trial was at least forty-eight hours to no more than one week after the pre-supplementation practice trial. One time trial was completed with each treatment, and each trial was separated by at least forty-eight hours to no more than one week to allow for a washout of the previous treatment. The number of days between each trial was kept consistent during all trials for each subject. This time was used to control for variability, detraining, fatigue, and to insure for an effective washout period since the half-life of nitrates is 5-8 hours in the body (Hord et al., 2009). The treatment order was randomly assigned and kept in a mostly double blind manner. Participants arrived at the lab three hours before each performance test and were placed in a seated position and allowed to rest quietly for fifteen minutes. Two hours and forty-five minutes before the warm-up, systemic blood pressure was measured at the right brachial artery. Two hours and thirty minutes before the warm-up, subjects were required to drink 70 ml of the treatment fluid. This time of 2.5 hours prior to the warm-up was used since previous studies have determined that the plasma nitrite and vasodilatory effects of dietary nitrates peak after 2.5-3 hours of ingestion of the nitrates (Kapil et al., 2010; Webb et al., 2008). Subjects were allowed to leave the lab provided that they did not consume any food or fluids or undergo any physical activity. They were required to return to the lab 2 hours later to undergo a 15 minute seated resting period before blood pressure measurements were taken. Fifteen minutes before the warm-up, resting systemic blood pressure was measured again, and instructors and subjects prepared for the warm-up. The warm-up lasted ten minutes and began at 50 W below the starting work rate



of each subject's  $\text{VO}_2$  max test (Morris et al., 2011) on the same Lode cycle ergometer used in the pre-supplementation period. After one minute, the power output was increased by 10 W and continued to increase by 10 W for five minutes (Morris et al., 2011). Subjects then remained at the wattage reached (starting wattage of the  $\text{VO}_2$  max test) for five more minutes (Morris et al., 2011). Subjects then rested for five minutes while the equipment was prepared for the performance test, and another systemic blood pressure measurement was taken at the end of the five minute rest period. The Lode ergometer was placed in a mode to allow for manual adjustments of work rates. This set up was done to simulate an actual time trial race. Following the five minute rest period and blood pressure measurement, subjects became accustomed to breathing the hypoxic gas for three minutes while sitting on the cycle ergometer. Eighteen minutes past the warm-up start time, subjects started motionless in a gated start style waiting on a cue from the lab technician to begin the performance test under the hypoxic conditions. Subjects were able to change work rates as desired. Similar to the practice trial, subjects received feedback only on their work completed; time, speed, and power output were withheld. The investigators gave verbal encouragement similar to that in the pre-supplementation trial.  $\text{VO}_2$  and RER were monitored with the metabolic cart in the same way as the  $\text{VO}_2$  max test. Blood oxygen saturation, elapsed time, and heart rate were recorded after 0.0, 0.25, 0.50, 0.75, and 1.00 of the total kilojoules were completed. After completion of the trial, subjects were allowed to remove the mouth piece and nose clip and cool down manually adjusting the work rate.

### **Means of data collection.**

***Pre-supplementation measurements.*** At the first visit before the VO<sub>2</sub> max test, the same investigator measured the height and weight of each subject using the standard beam scale (Health o Meter Professional Standard Beam Scale with Height Rod 402KL, North Shore Care Supply, Northbrook, IL) in centimeters and kilograms, respectively. During the VO<sub>2</sub> max test, expired respiratory gases were collected continuously, and the gas concentrations were analyzed for oxygen consumption and carbon dioxide production by the metabolic cart (Parvomedics Truemax 2400, Consentius Technologies, Sandy, UT). Oxygen consumption (VO<sub>2</sub>), heart rate, and respiratory exchange ratio (RER) were measured and averaged over fifteen second intervals by the metabolic cart. VO<sub>2</sub> max was measured as the highest reported oxygen consumption value with the fulfillment of three criteria: a plateau in oxygen consumption with an increase in work rate, an RER greater than 1.15, and a heart rate similar to the age-estimated maximum heart rate for the subject (220-age) (Bassett & Howley, 2000). The metabolic cart was calibrated according to the manual before each use. The age, height, weight, and maximum oxygen consumption for the nine subjects were measured and reported as (mean  $\pm$  SD)

***Supplementation measurements.*** Before each performance trial, systemic blood pressure of the right brachial artery was measured using the same manual aneroid sphygmomanometer and stethoscope (Prosphyg 775, Series sphygmomanometer and Adscope stethoscope, American Diagnostic Corporation, Hauppauge, NY) by the same investigator for each subject. Systolic (SP) and diastolic (DP) blood pressure were determined by the sphygmomanometer and used to estimate the mean arterial pressure

(MAP) using the standard equation:  $MAP = \frac{2}{3} DP + \frac{1}{3} SP$ . Blood pressure was measured three times: before the supplement was ingested, two hours and fifteen minutes after the supplement was ingested before the warm-up, and two hours and forty minutes after the supplement was ingested after the warm-up. Blood oxygen saturation and heart rate were measured after 0.00, 0.25, 0.50, 0.75 and 1.00 of the total kilojoules were completed using a pulse oximeter that was clipped to the subject's earlobe (Nonin Digital Handheld Pulse Oximeter, 8500; Nonin Medical Inc., Plymouth, MN). Completion time was measured using the timer on the metabolic cart at the end of each segment and at the end of each performance time trial (0.25, 0.50, 0.75, and 1.00 of the total kilojoules completed). Average work rate (W) for each segment and time trial was determined based on the distance completed (Lode cycle ergometer: 16 kJ/ 1 km) and the time to complete each segment and total time trial. Average oxygen consumption (ml/min) was determined for each segment (0.25, 0.50, 0.75, and 1.00) and averaged to calculate average oxygen consumption for each time trial. Mean cycling economy was determined as average work rate / average oxygen consumption ( $J \cdot min^{-1} / ml \cdot min^{-1}$ ) during each time trial. All values were reported as mean  $\pm$  SD.

**Statistical analysis.** Two-Way Repeated Measures Group by Treatment by Time Analysis of Variance (ANOVA) was used to assess the effects of treatment (BR, PL, and CO), time, and the interaction of treatment and time on systolic blood pressure, diastolic blood pressure, and mean arterial pressure at three time points (before ingestion, after ingestion before the warm-up, and after the warm-up), on blood oxygen saturation at 0.00, 0.25, 0.50, 0.75, and 1.00 of the total kilojoules completed, and on completion time and work rate for 0.25, 0.50, 0.75, and 1.00 of the total kilojoules completed. One-Way Repeated

Measures Group by Treatment Analysis of Variance was used to assess the effects of treatments on the percent change in systolic, diastolic, and mean arterial blood pressure from before ingestion to after ingestion before the warm-up and from before ingestion to after the warm-up and on average time trial completion time, average work rate, average oxygen consumption, and average cycling economy of the time trial cycling tests. Following a significant F-ratio, specific differences between treatments were explored using Sidak post-hoc analyses using Cohen's  $F^2$ . Statistical significance was set at  $P \leq 0.05$ . A Paired Samples T-test was used to compare the percent difference in time trial completion time between BR and CO to the percent difference in time trial completion time between PL and CO. A Pearson r correlation was conducted to examine the relationship between average oxygen consumption and time trial completion time. With relatively small sample size, rather large differences may not reach statistical significance and result in type II error with other secondary variables. Therefore, effect sizes (partial  $\eta^2$ , which represents the proportion of total variation attributable to the factor, partialing out other factors from the total nonerror variation) are reported as an additional statistical parameter to aid the reader in interpretation of the findings. All statistical analysis was performed using statistical analysis software (SPSS, Version 20.0, SPSS Inc., Chicago, IL).

## Results

**Subjects.** Nine healthy competitive male cyclists and triathletes (mean  $\pm$  SD: age 22  $\pm$  4 years, height 181  $\pm$  8 cm, body mass 73.80  $\pm$  8.3 kg, VO<sub>2</sub>max 66.7  $\pm$  8.6 ml/kg/min) participated in the study. The beetroot juice (BR), placebo (PL), and control (CO) supplementation was well tolerated with no reported harmful side effects. Hypoxic gas exposure was well tolerated by all subjects. Data was scanned for compliance with the diet, physical activity, and sleep requirements. Although there were slight variations in physical activity and diet, the data moved in no direction that suggested an effect of compliance on exercise performance.

**Blood pressure.** Blood pressure measurements were taken before ingestion (before ingestion), 2 hours and 15 minutes after ingestion before the warm-up (after ingestion before the warm-up), and 2 hours and 45 minutes after ingestion after the warm-up (after the warm-up). Systolic blood pressure, diastolic blood pressure, and mean arterial pressure for each time point and each treatment are shown in Table 1 on page 61. No significant main effects due to treatment were observed at any of the measurement times in systolic blood pressure (BR vs PL,  $P=0.24$ ; BR vs CO,  $P=0.35$ ; PL vs CO,  $P=0.95$ ; Effect Size=0.25, Observed Power=0.46), diastolic blood pressure (BR vs PL,  $P=0.99$ ; BR vs CO,  $P=0.99$ ; PL vs CO,  $P=0.98$ ; Effect Size=0.01, Observed Power=0.06), and mean arterial pressure (BR vs PL,  $P=0.96$ ; BR vs CO,  $P=0.62$ ; PL vs CO,  $P>0.99$ ; Effect Size=0.04, Observed Power=0.83). Significant main effects due to time were observed in systolic blood pressure ( $P<0.01$ , Effect Size=0.70, Observed Power>0.99) and mean arterial pressure ( $P=0.02$ , Effect Size=0.51, Observed Power=0.77). No significant main effects due to time were observed in diastolic

blood pressure ( $P=0.07$ , Effect Size=0.28, Observed Power=0.52). Significant increases in blood pressure from before ingestion to after the warm-up were revealed in systolic blood pressure under the PL treatment (PL,  $P=0.01$ ); however, systolic blood pressure did not have significant increases under the BR and CO treatments (BR,  $P=0.26$ ; CO,  $P=0.10$ ). Diastolic blood pressure and mean arterial pressure increased, but these increases were not statistically significant (Diastolic: BR,  $P=0.05$ ; PL,  $P=0.98$ ; CO,  $P=0.65$ ; MAP: BR,  $P=0.06$ ; PL,  $P=0.26$ ; C,  $P=0.28$ ). Significant increases in blood pressure from after ingestion before the warm-up to after the warm-up were observed in systolic blood pressure under the BR and PL treatments (BR,  $P=0.02$ ; PL,  $P=0.01$ ) and in mean arterial pressure under the BR treatment (BR,  $P=0.04$ ); but, these increases were not significant in systolic blood pressure under the control treatment (CO,  $P=0.07$ ) and in mean arterial pressure under the PL and CO treatments (PL,  $P=0.07$ ; CO,  $P=0.45$ ). These increases were also not significant in diastolic blood pressure under the BR and CO treatments and resulted in no change under the PL treatment (BR,  $P=0.15$ ; PL,  $P>0.99$ ; CO,  $P=0.99$ ). Non-significant decreases from before ingestion to after ingestion before the warm-up were observed in systolic blood pressure under the BR, PL, and CO treatments (BR,  $P=0.08$ ; PL,  $P=0.32$ ; CO,  $P=0.59$ ) and mean arterial pressure under the PL treatment (PL,  $P=0.93$ ). Non-significant increases from before ingestion to after ingestion before the warm-up were observed in diastolic blood pressure under the BR, PL, and CO treatments (BR,  $P=0.50$ ; PL,  $P=0.95$ ; CO,  $P=0.38$ ) and in mean arterial pressure under the BR and CO treatments (BR,  $P=0.99$ ; CO,  $P=0.61$ ). No significant interaction between treatment and time was observed in systolic blood pressure ( $P=0.74$ , Effect Size=0.06, Observed Power=0.15), diastolic blood pressure ( $P=0.09$ , Effect Size=0.22, Observed Power=0.58), or mean arterial pressure ( $P=0.36$ , Effect Size=0.12, Observed

Power=0.31). Percent change in blood pressure from before ingestion to after ingestion before the warm-up was observed to be non-significant for each of the three treatments in systolic blood pressure (BR vs PL,  $P=0.93$ ; BR vs CO,  $P=0.61$ ; PL vs CO,  $P=0.94$ ; Effect Size=0.07, Observed Power=0.16), diastolic blood pressure (BR vs PL,  $P=0.72$ ; BR vs CO,  $P=0.98$ ; PL vs CO,  $P=0.72$ ; Effect Size=0.09, Observed Power=0.16), and mean arterial pressure (BR vs PL,  $P=0.93$ ; BR vs CO,  $P=0.99$ ; PL vs CO,  $P=0.66$ ; Effect Size=0.06, Observed Power=0.11). Percent change in blood pressure from before ingestion to after the warm-up was observed to be non-significant for each of the three treatments in systolic blood pressure (BR vs PL,  $P=0.66$ ; BR vs CO,  $P=0.87$ ; PL vs CO,  $P=0.97$ ; Effect Size=0.08, Observed Power=0.15), diastolic blood pressure (BR vs PL,  $P=0.20$ ; BR vs CO,  $P=0.31$ ; PL vs CO,  $P=0.73$ ; Effect Size=0.27, Observed Power=0.49), and mean arterial pressure (BR vs PL,  $P=0.46$ ; BR vs CO,  $P=0.63$ ; PL vs CO,  $P=0.80$ ; Effect Size=0.17, Observed Power=0.29).

Table 1  
*Blood Pressure Responses to Treatments*

Blood Pressure Measurement (mmHg)		Beetroot Juice	Placebo	Control
<b>Systolic Blood Pressure</b>				
	Before Ingestion	123 ± 7	117 ± 4	118 ± 8
	After Ingestion Before Warm-Up	117 ± 9	113 ± 8	115 ± 10
	After Warm-Up	# 128 ± 7	*# 125 ± 7	125 ± 10
% Change From Before Warm-Up to After Ingestion Before Warm-Up		-5 ± 5 %	-3 ± 5 %	-2 ± 5 %
% Change From Before Warm-Up to After Warm-Up		4 ± 7 %	7 ± 5 %	6 ± 7 %
<b>Diastolic Blood Pressure</b>				
	Before Ingestion	74 ± 7	78 ± 9	76 ± 8
	After Ingestion Before Warm-Up	78 ± 6	79 ± 9	79 ± 6
	After Warm-Up	84 ± 6	79 ± 12	80 ± 8
% Change From Before Warm-Up to After Ingestion Before Warm-Up		5 ± 11 %	1 ± 5 %	4 ± 9 %
% Change From Before Warm-Up to After Warm-Up		14 ± 14 %	1 ± 11 %	6 ± 16 %
<b>Mean Arterial Pressure</b>				
	Before Ingestion	90 ± 6	91 ± 5	90 ± 6
	After Ingestion Before Warm-Up	91 ± 6	90 ± 8	91 ± 5
	After Warm-Up	# 98 ± 6	95 ± 8	95 ± 6
% Change From Before Warm-Up to After Ingestion Before Warm-Up		1 ± 6 %	-1 ± 5 %	1 ± 3 %
% Change From Before Warm-Up to After Warm-Up		9 ± 10 %	4 ± 6 %	6 ± 9 %

\* $P < 0.05$  Compared to before ingestion for that treatment.

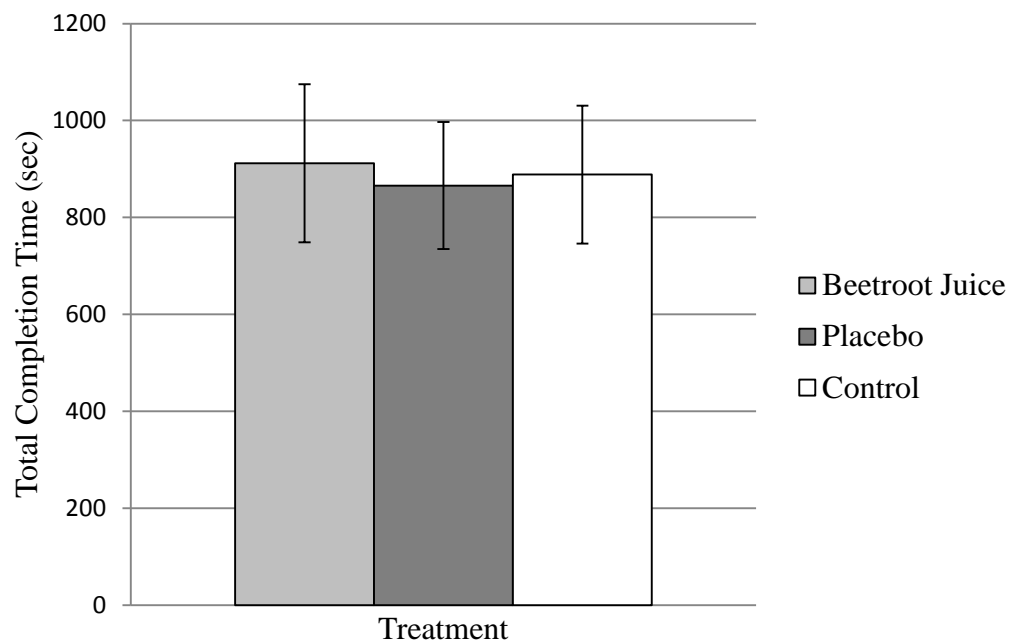
# $P < 0.05$  Compared to after ingestion before the warm-up for that treatment.

All values: Mean ± SD.



### **Time trial results.**

**Performance.** The data was tested for an order effect. No significant differences were found between the three time applications of the time trial tests (Test 1,  $897.11 \pm 153.81$  sec; Test 2,  $873.22 \pm 140.59$  sec; Test 3,  $895.67 \pm 146.20$  sec) (1 vs 2,  $P=0.83$ ; 1 vs 3,  $P>0.99$ ; 2 vs 3,  $P=0.35$ ; Effect Size=0.06, Observed Power=0.10). Results for total completion time based on treatment are displayed in Figure 1 on page 63. No significant differences in time trial completion times were observed when comparing the three treatments (BR vs PL,  $P=0.33$ ; BR vs CO,  $P=0.87$ ; PL vs CO,  $P=0.50$ ; Effect Size=0.16, Observed Power=0.28). Completion time for each treatment was: BR:  $911.78 \pm 163.07$  sec, PL:  $865.78 \pm 131.02$  sec, and CO:  $888.44 \pm 142.32$  sec. Results for completion time for each segment under each treatment are displayed in Table 2 on page 64. There also were no differences observed in performance during any of the time trial segments due to treatment; however, there was a significant difference due to the portion of the time trial completed. There was a significant increase in segment completion time for the 0.00-0.25 segment compared to all other segments in the BR treatment only (BR: 0.00-0.25 vs 0.25-0.50,  $P<0.01$ ; vs 0.50-0.75,  $P=0.03$ ; vs 0.75-1.00,  $P=0.02$ ; Effect Size=0.57, Observed Power>0.99). There was no significant effect due to the interaction of treatment and portion of the time trial completed ( $P=0.11$ , Effect Size=0.24, Observed Power=0.45). Finally, no significant differences were observed when comparing the percent difference in completion time between BR and CO to the percent difference in completion time between PL and CO ( $2.32 \pm 10.57\%$  and  $-2.43 \pm 5.42\%$ ; respectively,  $P=0.12$ , Effect Size=0.28, Observed Power=0.34).



*Figure 1.* Time trial completion time.  
All values: Mean  $\pm$  SD.

Table 2  
*Completion Times for Each Segment of the Time Trial*

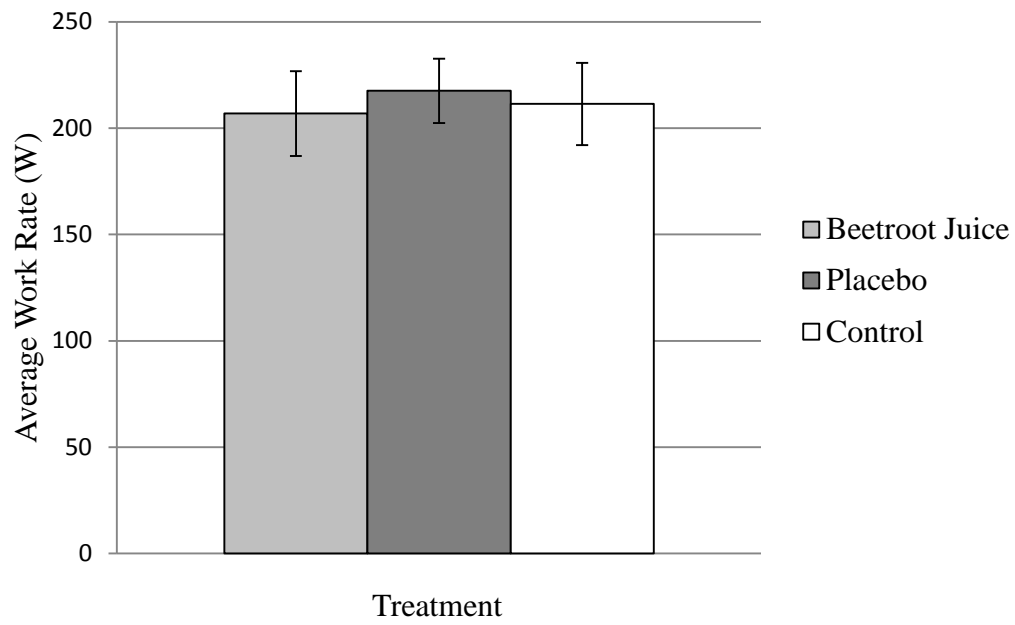
Segment	Beetroot Juice	Placebo	Control
0.00-0.25	* 253.33 ± 39.65 sec	232.00 ± 50.21 sec	222.89 ± 44.75 sec
0.25-0.50	221.89 ± 45.56 sec	206.11 ± 31.67 sec	215.22 ± 40.45 sec
0.50-0.75	223.56 ± 50.39 sec	214.44 ± 40.69 sec	227.56 ± 42.14 sec
0.75-1.00	213.00 ± 35.20 sec	213.22 ± 38.61 sec	222.78 ± 31.72 sec

\* P<0.05. From all other segments for BR treatment only.  
 All values: Mean ± SD.

Results for average work rate based on treatment are displayed in Figure 2 on page 66.

The treatment did not have significant effects on the average work rate for each time trial (BR vs PL,  $P=0.27$ ; BR vs CO,  $P=0.91$ ; PL vs CO,  $P=0.33$ ; Effect Size=0.18, Observed Power=0.32).

Average work rate under each treatment was as follows: BR,  $206.8 \pm 20.0$  W; PL,  $217.5 \pm 15.1$  W; and CO,  $211.4 \pm 19.3$  W. Results for work rate for each segment completed of the time trial under each treatment are displayed in Table 3 on page 67. There were also no differences observed in work rate during any of the time trial segments due to treatment; however, there was a significant difference due to the portion of the time trial completed. There was a significant decrease in work rate for the 0.00-0.25 segment compared to all other segments in the BR treatment only (BR: 0.00-0.25 vs 0.25-0.50,  $P=0.01$ ; vs 0.50-0.75,  $P=0.03$ ; vs 0.75-1.00,  $P=0.02$ ; Effect Size=0.64, Observed Power=0.98). There was no significant effect due to the interaction of treatment and portion of the time trial completed ( $P=0.13$ , Effect Size=0.23, Observed Power=0.75).



*Figure 2.* Work rate during the time trial.  
All values: Mean  $\pm$  SD.

Table 3  
*Work Rates for Each Segment of the Time Trial*

Segment	Beetroot Juice	Placebo	Control
0.00-0.25	* 184.71 ± 13.37 W	206.07 ± 37.19 W	219.61 ± 30.12 W
0.25-0.50	212.11 ± 23.64 W	225.93 ± 19.68 W	218.18 ± 25.73 W
0.50-0.75	213.06 ± 27.00 W	219.94 ± 22.25 W	207.29 ± 20.83 W
0.75-1.00	217.46 ± 23.65 W	218.22 ± 28.25 W	207.19 ± 21.05 W

\* P<0.05. From all other completion points for BR treatment only.  
 All values: Mean ± SD.

**Physiological.** Treatment had no significant effect on arterial blood oxygen saturation at any portion of the trial completed during the time trial (BR vs PL,  $P=0.69$ ; BR vs CO,  $P>0.99$ ; PL vs CO,  $P=0.83$ ; Effect Size=0.05, Observed Power=0.11). There was a significant effect for the measurement point with regard to blood oxygen saturation ( $P<0.01$ , Effect Size=0.95, Observed Power>0.99). Oxygen saturations at each completion point for each treatment are displayed in Table 4 on page 69. Blood oxygen saturation was significantly higher at the starting point (0.00) of the time trial versus all other completion points under all three treatments (BR: 0.00 vs 0.25, 0.50, 0.75, 1.00  $P<0.01$ ; Effect Size=0.88, Observed Power>0.99; PL: 0.00 vs 0.25, 0.50, 0.75, 1.00  $P<0.01$ ; Effect Size=0.92, Observed Power>0.99; CO: 0.00 vs 0.25, 0.50, 0.75, 1.00  $P<0.01$ ; Effect Size=0.91, Observed Power>0.99). Significant decreases in blood oxygen saturation were also observed between the 0.25 completion point and the 1.00 completion point under the PL treatment ( $P=0.01$ ). No significant interaction between treatment and portion of time trial completed ( $P=0.27$ , Effect Size=0.15, Observed Power=0.31) was observed.

Table 4  
*Arterial Blood Oxygen Saturation of Cyclists During the Time Trials*

Completion Point	Beetroot Juice	Placebo	Control
0.00	* 93 ± 3 %	* 94 ± 3 %	* 94 ± 4 %
0.25	76 ± 4 %	# 76 ± 5 %	76 ± 3 %
0.50	74 ± 3 %	74 ± 6 %	74 ± 4 %
0.75	73 ± 3 %	71 ± 4 %	73 ± 4 %
1.00	74 ± 7 %	70 ± 3 %	73 ± 5 %

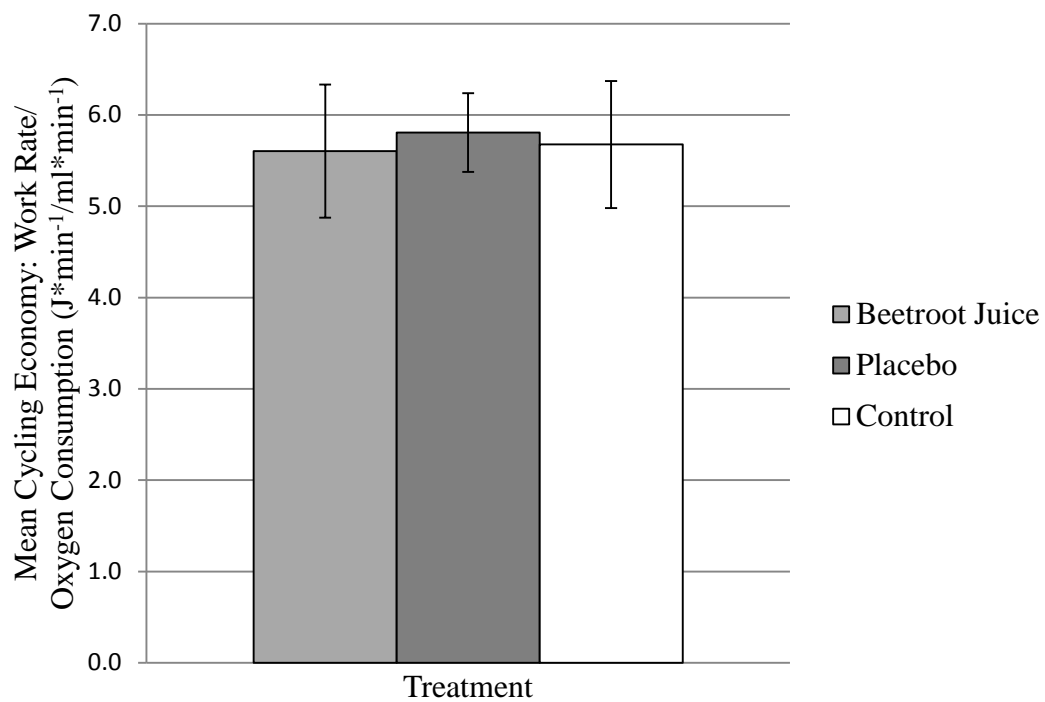
\*  $P < 0.05$ . From all other completion points for that treatment.

#  $P < 0.05$ . From 1.00 completion point of the same treatment.

All values: Mean ± SD



Additionally, there were no significant effects due to treatment observed in average oxygen consumption during the time trial between any of the three treatments (BR vs PL,  $P=0.99$ ; BR vs CO,  $P=0.99$ ; PL vs CO,  $P=0.99$ ; Effect Size=0.01, Observed Power=0.58). Average oxygen consumption under each treatment was as follows: BR,  $2230.7 \pm 211.9$  ml/min; PL,  $2252.5 \pm 140.5$  ml/min; and CO,  $2245.8 \pm 154.9$  ml/min. Average oxygen consumption was not strongly correlated to time trial completion time ( $r=0.2$ ,  $P=0.32$ ). Finally, there were no significant treatment effects on mean cycling economy (Work Rate / Oxygen Consumption ( $\text{J} \cdot \text{min}^{-1} / \text{ml} \cdot \text{min}^{-1}$ )) during the time trial between any of the three treatments (BR vs PL,  $P=0.90$ ; BR vs CO,  $P> 0.99$ ; PL vs CO,  $P=0.77$ ; Effect Size=0.04, Observed Power=0.09). Results for mean cycling economy based on treatment are displayed in Figure 3 on page 71. Mean cycling economy for each treatment was as follows: BR,  $5.6 \pm 0.7 \text{ J} \cdot \text{min}^{-1} / \text{ml} \cdot \text{min}^{-1}$  of oxygen consumed; PL,  $5.8 \pm 0.4 \text{ J} \cdot \text{min}^{-1} / \text{ml} \cdot \text{min}^{-1}$  of oxygen consumed; and CO,  $5.7 \pm 0.7 \text{ J} \cdot \text{min}^{-1} / \text{ml} \cdot \text{min}^{-1}$  of oxygen consumed.



*Figure 3.* Cycling economy during the time trial.  
All values: Mean  $\pm$  SD.

## Test of Research Hypotheses

1. Systolic blood pressure, diastolic blood pressure, and mean arterial pressure would be decreased under the beetroot juice treatment compared to the placebo and control.  
The hypothesis was rejected. No significant differences in systolic blood pressure, diastolic blood pressure, and mean arterial pressure were observed due to treatment ( $P>0.05$ ).
2. Time trial cycling performance time would be decreased under the beetroot juice treatment compared to the placebo and control. The hypothesis was rejected. No significant difference in completion times was observed due to treatment ( $P>0.05$ ).
3. Arterial blood oxygen saturation would be increased under the beetroot juice treatment compared to the placebo and control. The hypothesis was rejected. No significant difference in blood oxygen saturation was observed due to treatment ( $P>0.05$ ).

## Discussion

The purpose of this study was to determine the effect of short-term red beetroot juice supplementation on systemic blood pressure, exercising arterial blood oxygen saturation, and acute aerobic (approximately 200 kJ) time trial performance during acute hypoxic exposure (simulated high altitude (4300 m)) in male club level cyclist and triathletes. The results showed that beetroot juice did not have a significant effect on systemic blood pressure, exercising arterial blood oxygen saturation, and time trial cycling performance time during hypoxic exposure compared to placebo and control.

**Blood pressure.** Blood pressure was taken before ingestion (before ingestion), 2 hours and 15 minutes after ingestion before the warm-up (after ingestion before the warm-up), and 2 hours and 45 minutes after ingestion at the end of a 5 minute rest period following the warm-up (after the warm-up). It was hypothesized that beetroot juice supplementation would result in a decrease in systolic blood pressure, diastolic blood pressure, and mean arterial pressure; however, acute beetroot juice supplementation did not have an effect on systolic blood pressure, diastolic blood pressure, and mean arterial pressure when compared to placebo and control in the current study. This hypothesis was rejected. There was a statistically significant observed increase in systolic blood pressure under the beetroot and placebo treatments and in mean arterial pressure under the beetroot treatment when comparing the measurement points from after ingestion before the warm-up to after the warm-up. A rise in systolic blood pressure is a typical response during aerobic exercise (Filipovsky, Ducimetiere, & Safar, 1992). Systolic blood pressure also rose in the control group, and mean arterial pressure also rose in the placebo and control groups, but these

changes were not statistically significant, possibly because of large variance in the data. An increase in blood pressure from before ingestion to after the warm-up would have also been expected. All systolic blood pressures rose over this period, but possibly due to large standard deviations, the only blood pressure increase that was found statistically significant was in systolic blood pressure under the placebo treatment. Additionally, no significant differences were observed when comparing before ingestion to after ingestion before the warm-up. Systolic blood pressure decreased under all treatments with the largest decrease observed in the beetroot juice treatment group, but these decreases did not result in statistical significance under any treatment. Some of the systolic pressure reduction in all treatment groups could be due to the majority of the subjects deciding to remain seated in the lab following the ingestion of the treatment and before the pre-warm-up blood pressure measurement, but large standard deviations and several limitations could have resulted in observing the lack of significance in the beetroot juice treatment group.

One limitation was that blood pressure in this study was only measured one time at the three measurement points. In addition, while all measurements for each subject were done by the same investigator, the use of a manual blood pressure cuff could have resulted in a larger source of variability than using an automatic device. Previous studies have reported reductions in systemic blood pressure due to the beetroot juice treatment even though results varied (Bailey et al., 2009; Kapil et al., 2010; Lansley et al., 2011; Vanhatalo et al., 2010; Webb et al., 2008). Both Kapil et al. (2010) and Lansley et al. (2011) observed decreases in systolic blood pressure but found no significant change in diastolic blood pressure and mean arterial pressure after acute (2.5 hours) beetroot juice supplementation. Bailey et al. (2009) observed similar pressure results to Kapil et al. (2010) and Lansley et al. (2011) after

ingestion each day following a chronic (4-6 days) supplementation pattern with beetroot juice compared to placebo. Vanhatalo et al. (2010) observed decreases in systolic blood pressure, diastolic blood pressure, and mean arterial pressure after acute (2.5 hours) beetroot juice supplementation. Webb et al. (2008) observed similar results to Vanhatalo et al. (2010) and reported that the systolic blood pressure decreases were significant after 2.5 hours post beetroot juice ingestion, while the diastolic blood pressure and mean arterial pressure decreases were not significant until the 3 hour measurement point post-ingestion. All of the previous studies recorded measurements in triplicate or quadruplicate and used an automatic sphygmomanometer which may have resulted in lower variability in measurements (Bailey et al., 2009; Kapil et al., 2010; Lansley et al., 2011; Vanhatalo et al., 2010; Webb et al., 2008). Furthermore, the time of measurement in this study may have been a reason why significance was not observed. While other studies have shown that systemic blood pressure was reduced following beetroot juice ingestion, peak plasma nitrite, cGMP levels, and therefore blood pressure reduction effects peaked between 2.5-3 hours post beetroot juice ingestion (Kapil et al., 2010; Webb et al., 2008). In this study, blood pressure could have been measured too early in hopes of timing the peak effects of the nitrates to occur during the exercise performance trial. The nitrates in red beetroot juice did not reduce systemic blood pressure suggesting that systemic vasodilation did not occur in response to beetroot juice supplementation in this study.

### **Time trial results.**

**Performance.** All subjects completed a 200 kJ time trial following the ingestion of each treatment. The objective of the test was to complete it as quickly as possible. Total time to completion and the times to complete the first, second, third, and fourth quarters of the tests were measured. Time to completion measurements were used to calculate average work rate for each quarter of the time trial and for the entire time trial. The results revealed that under the beetroot juice treatment, mean time to completion was significantly slower and mean power output was significantly lower in the 0.00-0.25 segment when compared to the other treatments. No other significant differences were observed in time trial performance. It was hypothesized that beetroot juice supplementation would result in a decrease in the amount of time it took to complete the time trial compared to placebo and control; however, no effects of supplementation on exercise performance were observed. This hypothesis was rejected. The significant difference in the time to completion of the 0.00-0.25 segment was primarily due to the poor performance of three subjects. Two subjects had the beetroot juice treatment first and therefore poor pacing could be a result of unfamiliarity with this type of cycle ergometer. The other had the beetroot juice treatment last. Observed power for the time trial performance analysis was low (0.28), but the inconsistent responses in time trial performance of the subjects during the beetroot juice trial, suggest no treatment effect was present.

Previous studies of beetroot juice supplementation on aerobic exercise performance have yielded mixed results (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010). Lansley et al. (2011) reported significant improvements in time trial performance at both a 4 km and 16.1 km distance (2.8% and 2.7% decrease in completion time, respectively)

compared to the placebo treatment. They also reported significant improvements in mean power output of 5% and 6% for the 4 km and 16.1 km time trial, respectively. This shows evidence of beetroot juice improving the performance of an aerobic cycling test ranging from approximately 5 to 30 minutes. The exercise test in this study falls in the middle of that range at approximately 15 minutes. Furthermore, beetroot juice has been shown to improve other types of cycling aerobic exercise tests. Bailey et al. (2009) found a significant increase in time to task failure during a severe intensity constant work rate cycling test to exhaustion after 6 days of beetroot juice supplementation that was not observed under the placebo treatment. Additionally, Vanhatalo et al. (2010) found that after more than 5 days of beetroot juice supplementation, time to exhaustion and therefore, peak power output, in an incremental to maximum exercise cycling test was improved during the test on the 15<sup>th</sup> day of supplementation, but Vanhatalo et al. (2010) did not observe an improvement in time to exhaustion after acute beetroot juice supplementation (2.5 hours) or after 5 days of chronic beetroot juice supplementation. All of these previous studies tested beetroot juice supplementation under normoxic conditions.

The nitrates in beetroot juice have been shown to improve cycling performance in normoxia (Bailey et al., 2009; Lansley et al., 2011; Vanhatalo et al., 2010), and to have blood pressure reduction (Bailey et al., 2009; Kapil et al., 2010; Lansley et al., 2011; Vanhatalo et al., 2010; Webb et al., 2008) and vasodilatory effects in the systemic circulation (Kapil et al., 2010; Webb et al., 2008). These systemic pressure and vasodilatory effects are stimulated through a pathway in which the nitrates get converted first into nitrite and then into nitric oxide ultimately affecting cGMP signaling (Kapil et al., 2010; Webb et al., 2008). Hypoxic conditions induce reflexive pulmonary vasoconstriction which impairs aerobic exercise



performance (Hahn & Gore, 2001; Hsu et al., 2006; Martin et al., 2010; Richalet et al., 2005; Scherrer et al., 1996; Schoene, 2001; Sheel et al., 2010). While infused nitrite and inhaled nitric oxide have been shown to cause pulmonary vasodilation under hypoxic conditions at rest (Ingram et al., 2010; Scherrer et al., 1996), dietary nitrates, such as red beetroot juice, have not been tested for pulmonary vasodilatory effects. Hypoxic aerobic exercise performance has also not been analyzed under beetroot juice supplementation. This study was designed to monitor indirect indicators of pulmonary vasculature tone and ultimately test aerobic exercise performance during beetroot juice supplementation under hypoxic conditions.

Supplementation of pulmonary vasodilators, phosphodiesterase-5 (PDE-5) inhibitors have been shown to improve aerobic cycling performance in hypoxic environments (Bailey et al., 2009; Ghofrani et al., 2004; Hsu et al., 2006; Richalet et al., 2005). PDE-5 inhibitors have been shown to stimulate these pulmonary vasodilatory effects via cGMP signaling (Richalet et al., 2005; Zuckerbraun et al., 2011). In a study with similar acute hypoxic gas exposure (12.8% oxygen) to this current study (~13% oxygen), Hsu et al. (2006) reported an increase in a 6 km time trial performance immediately following 30 minutes of cycling at a constant work rate of 55% peak power output with the use of PDE-5 supplementation compared to placebo supplementation; however, supplementation did not improve performance under sea level conditions (Hsu et al., 2006). Additionally, Richalet et al. (2005) reported increased performance on an incremental to maximum cycling test when tested on days 2 and 5 of an actual ascent to a similar altitude simulated in this study of 4350 m. Likewise, Ghofrani et al. (2004) observed increased exercise tolerance during an incremental to maximum cycling test with PDE-5 inhibitor supplementation under both acute

hypoxic exposure (of a gas containing 10% oxygen) and chronic hypoxic exposure (after 6 days of acclimitization at 5,245 m) compared to placebo. There is a possibility that the nitrates in red beetroot juice may have a different mechanism of performance improvement than PDE-5 inhibitors and may or may not be effective with pulmonary vasoconstriction or aerobic exercise in hypoxia. In conclusion, the nitrates in red beetroot juice did not improve aerobic cycling performance at hypoxia by decreasing time trial time and improving work rate in this study.

**Physiology.** It was hypothesized that beetroot juice supplementation would result in increased arterial blood oxygen saturation levels during the time trial compared to placebo and control. Based on the results from this study, acute beetroot juice supplementation did not have an effect on arterial blood oxygen saturation at any completion point throughout the time trial when compared to the placebo and control treatments. This hypothesis was rejected. The only significant effect on arterial blood oxygen saturation observed was due to the measurement point during the time trial. Blood oxygen saturation was significantly higher at the starting point of the time trial than at all other completion points of the time trial for each treatment. This was expected since blood oxygen saturation decreases with exercise as increasing amounts of oxygen are offloaded from the blood to support the increasing metabolic demands of the tissues especially seen during exercise at hypoxia (Banchero et al., 1966; Bender et al., 1989). Oxygen saturation remained relatively stable for the duration of the time trial in all treatments with the exception of the placebo treatment which showed a small but significant drop at the 1.00 completion point compared to the 0.25 completion point. Overall, exercise blood oxygen saturation values under each treatment at a simulated

altitude of 4300 m in this study were similar to the 72.7 % mean blood oxygen saturation value Bender et al. (1989) reported from averaging blood oxygen saturation values at 5, 15, and 30 minutes during a constant cycling aerobic exercise test at 79%  $\text{VO}_{2\text{max}}$  after residing at 4300m, Pike's Peak (460 Torr Barometric Pressure) for 2 days.

This was the first study to our knowledge that measured blood oxygen saturation during beetroot juice supplementation and hypoxic aerobic exercise. Pulmonary vasodilatory (PDE-5 inhibitor) supplementation has been shown to increase arterial blood oxygen saturation during aerobic cycling exercise under acute (less than 2 hours) and chronic (days) hypoxic exposure (Ghofrani et al., 2004; Hsu et al., 2006; Ricart et al., 2005; Richalet et al., 2005). Infused and nebulized nitrite and inhaled nitric oxide have been shown to have pulmonary vasodilatory effects under hypoxic conditions in both animal and human tissue at rest (Ingram et al., 2010; Scherrer et al., 1996; Zuckerbraun et al., 2010). It has been shown in previous studies that dietary nitrates such as red beetroot juice get converted into nitrites and then to nitric oxide, ultimately reducing systemic blood pressure and causing systemic vasodilation (Bailey et al., 2009; Kapil et al., 2010; Lansley et al., 2011; Vanhatalo et al., 2010; Webb et al., 2008). Vascular tone and pressure has not been measured in the pulmonary system during previous beetroot supplementation studies. Furthermore, the effects of beetroot juice supplementation on the pulmonary vascular system have not been examined under hypoxic exposure. Pulmonary vasodilation has been linked to increases in blood oxygen saturation and blood flow (stroke volume, ejection fraction, cardiac output) (Ghofrani et al., 2004; Hsu et al., 2006; Ricart et al., 2005; Richalet et al., 2005). Therefore increases in blood oxygen saturation and blood flow could suggest the presence of pulmonary vasodilation. Since pulmonary vasculature tone and blood flow could not be

measured in this study, arterial blood oxygen saturation was our only method that could suggest the state of pulmonary vascular tone. In this study, beetroot juice had no significant effect on blood oxygen saturation levels; therefore, beetroot juice may be improving performance at normoxia (seen in previous studies) only through mechanisms other than pulmonary vasodilation. In conclusion, the nitrates in red beetroot juice did not improve the arterial blood oxygen desaturation seen during aerobic exercise under hypoxic conditions in this study which makes no suggestion of the presence of pulmonary vasodilation.

Acute beetroot juice supplementation did not have an effect on average oxygen consumption ( $\text{VO}_2$ ) during the time trial when compared to the placebo and control treatments in the current study. Additionally, there was also no observed correlation between average oxygen consumption and time trial completion time. Lansley et al. (2011) observed no differences in average  $\text{VO}_2$  throughout each time trial between beetroot juice supplementation and placebo supplementation for both 4 km and 16.1 km time trials under normoxic conditions. Furthermore, both Bailey et al. (2009) and Vanhatalo et al. (2010) observed a decrease in pulmonary  $\text{VO}_2$ , reduced oxygen cost, during submaximal, moderate constant work rate cycling exercise after both acute (2.5 hours) and chronic (days) beetroot juice supplementation that was not present in placebo. Bailey et al. (2009) discovered significantly increased blood flow and reduced muscle fractional oxygen extraction (supply : utilization) with beetroot juice supplementation during a moderate intensity constant work rate test suggesting that muscle energy production became more efficient since oxygen consumption decreased during a moderate constant work rate bout. Bailey et al. (2009) reported a change in oxygen kinetics (the oxygen uptake slow component was reduced) after chronic beetroot juice supplementation (6 days) during the severe intensity constant work

rate test to exhaustion without an alteration in fractional oxygen extraction like that seen in the moderate intensity constant work rate test. The continued increase in oxygen consumption seen with heavy to severe constant work rate exercise was reduced so that it took longer to reach maximum oxygen consumption and exhaustion under beetroot juice supplementation compared to placebo (Bailey et al., 2009). Vanhatalo et al. (2010) observed no changes in overall oxygen consumption under beetroot juice supplementation compared to placebo for a ramp incremental to exhaustion cycling test after chronic supplementation.

Furthermore, in the current study, acute beetroot juice supplementation did not have an effect on mean cycling economy during the time trial when compared to the placebo and control treatments. This is in contrast to Lansley et al. (2011) whom observed significant increases in mean cycling economy, defined as mean power output / oxygen consumption ( $W/L/min$ ), for both the 4 km and 16.1 km time trials (11% and 7%, respectively). Both Bailey et al. (2009) and Vanhatalo et al. (2010) observed a decrease in the functional gain ratio (oxygen consumed per minute/power output ( $ml \cdot min^{-1}/W$ )) in the moderate intensity constant work rate cycling tests. Furthermore, Bailey et al. (2009) observed a significant reduction in the overall gain ratio during the severe intensity constant work rate to exhaustion test following 6 days of beetroot juice supplementation compared to placebo. Like the Lansley et al. (2011) study, this also suggests beetroot juice helps improve cycling economy; however, Vanhatalo et al. (2010) observed that the functional gain ratio was not reduced in the ramp incremental to exhaustion cycling test until 15 days of beetroot juice supplementation. All of the previous exercise tests were completed under normoxic conditions. This is the first time, to our knowledge, that the effect of beetroot juice supplementation on oxygen consumption and cycling economy has been examined during

hypoxic aerobic exercise. In conclusion, acute beetroot juice supplementation did not have an effect on oxygen consumption and did not improve cycling economy in this study.

**Limitations.** Longer hypoxic exposure (a few days) would have more closely simulated the physiological hypoxic response to that of an actual race where the athlete ascends to an altitude of 4300m a few days before the competition, but we did not have the resources (time, money, location, or subject availability) to carry out this protocol. Furthermore, arterial blood oxygen saturation can only suggest the presence of pulmonary vasodilation. Due to the expense of a Doppler echocardiography instrument and the invasiveness of catheterization, we were not able to use these methods to gain a more accurate picture of the pulmonary vasculature. Also, subject compliance was a possible source of variation and error of true results. Even though subjects were instructed to maintain consistent diet, hydration, sleep, and physical activity behaviors and follow the diet and physical activity guidelines throughout the study, there was variability in these components. Based on the 24 hour logs, the subjects were compliant with the diet, hydration, and sleep requirements. About half of the subjects were compliant with the physical activity restrictions while the other half of the subjects were inconsistent with the intensity, duration, mode, and or pattern of outside physical activity performed throughout the study. This variability could have affected performance, but after examination of this specific data, it was determined that the data moved in no direction that suggested an effect of compliance on exercise performance. Additionally, even though the observed power was low, possibly due to a small sample size and larger variability in responses, there were no consistent changes in

performance, and this suggested that beetroot juice supplementation was not effective in improving oxygen saturation and performance under the parameters of the study.

### **Conclusion and Direction for Further Research**

Overall, this dose of acute beetroot juice supplementation did not have an effect on systemic blood pressure, exercising arterial blood oxygen saturation, and cycling time trial performance during the level of hypoxic exposure of this study. Therefore, dietary nitrate supplementation in the form of red beetroot juice may not be a prospective aid for not only helping athletes perform better at high altitude events, but also for helping military personnel and workers function better during aerobic exercise bout requirements of their job. Also, beetroot juice may not be a prospective aid to help people with pulmonary hypertension, pulmonary edema, and hypoxia related diseases function better during everyday activities. More testing is needed to more accurately and fully understand the pulmonary vasodilatory and performance enhancement potential of red beetroot juice supplementation during aerobic exercise at hypoxia.

This was the first time exercising blood oxygen saturation and aerobic exercise performance was analyzed during beetroot supplementation at hypoxia, so further replication of the study is needed to validate these results. Additionally, further studies should investigate the effects of different dosages of red beetroot juice supplementation and different levels of hypoxia to determine if the dose of beetroot juice in relation to the level of hypoxia used in this study was a reason for observing the lack of significance. Also, further investigation could be done to examine the effects in other populations with different exercise modality demands, training statuses, and sex than the subjects in this study such as military personnel, elite athletes, and female athletes, respectively. Furthermore, use of a



Doppler echocardiography instrument would help to give a more conclusive idea of the pulmonary vascular tone under each treatment in response to hypoxia than solely relying on the use of arterial blood oxygen saturation levels during exercise which can only suggest the presence of pulmonary vasodilation. Also, actually testing at 4300 m, following an ascent of no more than a few days, or residing in a hypobaric chamber for a few days, would allow for a more accurate and applicable determination of potential aerobic exercise performance effects for those athletes that just go up to altitude for the race only.

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## **Appendix A**

### Institutional Review Board Documents



## Request for Review of Human Participant Research

Appalachian Human Research Protection Program

IRB # (12-0255)

*Instructions: Complete and send the request form electronically to [irb@appstate.edu](mailto:irb@appstate.edu).*

*Note: checkboxes can be checked by putting an "x" in the box.*

### Section I: Study Description

1. Study Title: Effect of acute dietary nitrate supplementation on arterial blood oxygen saturation and time trial performance during acute hypoxic exposure in male cyclists and triathletes

2. Study Description: *Please describe briefly the objectives of the study with the purpose, research question and any relevant background information.*

Aerobic performance is impaired at high altitude due to reduced oxygen available to the body (aka hypoxia) caused by the reduced partial pressure of inspired oxygen ( $P_{iO_2}$ ) and hypoxia-induced pulmonary vasoconstriction. Hypoxic pulmonary vasoconstriction is a reflex mechanism whereby blood vessels in the lung constrict in response to hypoxia. This response reduces the oxygen transfer from the lung to the blood and results in lower oxygen delivery to the working muscle. Supplying vasodilators such as PDE-5 inhibitors has been shown to decrease hypoxic pulmonary vasoconstriction and improve blood oxygen levels and exercise performance in high altitude environments. Dietary nitrate supplementation has been shown to enhance vasodilation in the peripheral tissues such as exercising skeletal muscle, but their ability to alleviate hypoxic pulmonary vasoconstriction and improve exercise performance in hypoxia has not been evaluated. Therefore, the purpose of this study is to determine the effect of acute consumption of nitrate-rich red beetroot juice on arterial blood oxygen saturation and acute aerobic (approximately 200 kJ) time trial performance during acute hypoxic exposure (simulated high altitude (4300 m)) in male club level cyclists and triathletes.

3. Principal Investigator(s) and responsible faculty member if student is the PI: PI: Jennifer Arms, Faculty Co-Principle Investigator: Dr. David Morris  
Department(s): Health, Leisure, and Exercise Science

4. By submitting this request, the Principal Investigator (and responsible faculty member if PI is a student) accepts responsibility for ensuring that all members of the research team: 1) complete the required CITI training and any other necessary training to fulfill their study responsibilities, 2) follow the study procedures as described in the IRB approved application and comply with *Appalachian's Guidelines for the Review of Research Involving Human Subjects* and all IRB communication and 3) uphold the rights and welfare of all study participants.

The parties (i.e., the IRB and the Principal Investigator and responsible faculty member if PI is a student) have agreed to conduct this application process by electronic means, and this application is signed electronically by the Principal Investigator and by the responsible faculty member if a student is the PI.

My name and email address together constitute the symbol and/or process I have adopted with the intent to sign this application, and my name and email address, set out below, thus constitute my electronic signature to this application.

Jennifer Arms  
PI Name

armsjc@appstate.edu  
PI Email address

David Morris  
Responsible Faculty Name if PI is a student  
PI is a student

morrisdm@appstate.edu  
Responsible Faculty Email address if

5. Do you plan to publish or present off-campus?

☐ No ☒ Yes

6. Does this research involve any out-of-country travel?

☒ No ☐ Yes

7. Type of Research,  
check all that apply:

☐ Faculty Research ☒ Dissertation/Thesis/Honor's Thesis  
☐ Product of Learning ☐ Class Project – Course Number:  
☐ Educational Research Involving Normal Education Practices  
☐ Other: describe

8. Source of  
Funding

☐ Not Funded  
☐ Federally  
Funded

☐ Funds Awarded ☒ Funds Pending  
☐ University Funded: describe

If funds awarded/pending, provide sponsor name, Sponsored Programs number: Office of Student Research Grant, GSAS Research Grant, Cratis D. Williams Research Grant, NSCA GNC Nutritional Grant, NSCA Master's Grant.

*Attach a copy of the contract/grant/agreement.*

**9.** Is another institution engaged in the research (i.e., an agent of another institution will obtain informed consent, interact with participants to obtain information, or access private identifiable information about participants)?

☒ No    ☐ Yes    If yes, list institution(s) and whether that IRB will review or rely on the ASU IRB.

**10.** What, if any, relationship exists between the researcher(s) and agencies (e.g., schools, hospitals, homes) involved in the research? *Attach statement of approval (e.g., letter of agreement) from any agencies that will be involved with the research. None*

## Section II: Research Personnel

Enter each team member (including PI) in the table below. (*A member of the research team is defined as one who will: 1) access participants' private identifiable information, 2) obtain informed consent or 3) interact with participants.*)

Name	Role (e.g., PI, co-I, Research Assistant, Research Coord., Faculty Advisor, etc.)	Responsibilities: Select all that apply from the list of Responsibilities below (e.g., "a, b, c")	Receive IRB Correspondence (Y/N)?  If yes, provide preferred email address.
Jennifer Arms	PI	a, b, c, e, f, g, j, l, m	Yes armsjc@appstate.edu
David Morris	Co-PI	a, b, c, e, f, g, j, l, m	Yes morrisdm@appstate.edu

(**Note:** If you need additional room, you can add rows by going to right click, insert, and then insert rows below. Personnel changes made after IRB approval can be submitted via email with the above information.)

**Responsibilities:**

<b>a.</b> Screens potential participants	<b>h.</b> Conducts physical exams
<b>b.</b> Obtains Informed Consent	<b>i.</b> Collects biological specimens (e.g., blood samples)
<b>c.</b> Has access to identifiable data	<b>j.</b> Conducts study procedures
<b>d.</b> Administers survey	<b>k.</b> Dispenses medications
<b>e.</b> Conducts interviews	<b>l.</b> Supervises exercise
<b>f.</b> Enters subject data into research records	<b>m.</b> Educates participants, families, or staff
<b>g.</b> Analyzes data with identifiable information	<b>n.</b> Other: describe

**Note:** In some cases, expertise to perform study procedures (e.g., blood draws, interviewing participants about sensitive topics) should be documented by the IRB to show that risks to participants is minimized. The IRB uses the Research Personnel Form to document investigator expertise.

### **Section III: Conflict of Interest**

**1. Are there any known or potential conflicts of interest related to this research?**

*Conflict of interest relates to situations in which financial or other personal considerations may compromise or involve the potential/have the appearance for compromising an employee's objectivity in meeting University responsibilities including research activities.*

*Examples of conflicts of interest include but are not limited to: an investigator has equity in a business that conducts research in a related area; an investigator will receive an incentive/bonus based on the number or speed of enrollment or outcome of a study; or an investigator or family member is a consultant, holds an executive position or serves as a board member of the research sponsor or its holdings.*

☒ No ☐ Yes      If yes, describe and explain how participants will be protected from the influence of competing interests.

#### Section IV: Participant Population and Recruitment

1. Number of participants sought: 12

2. Targeted Participant Population (check all that apply):

<input checked="" type="checkbox"/>	Adults ( $\geq 18$ yrs old)	<input checked="" type="checkbox"/>	College Students (only 18 or older)
<input type="checkbox"/>	Minors ( $< 18$ yrs old) Age range:	<input type="checkbox"/>	College Students (under 18 may participate)
<input type="checkbox"/>	Minorities	<input type="checkbox"/>	Prisoners
<input type="checkbox"/>	Institutionalized Participants	<input type="checkbox"/>	Cognitively or emotionally impaired
<input type="checkbox"/>	Inpatient participants	<input type="checkbox"/>	Non-English speaking
<input type="checkbox"/>	Outpatient participants	<input type="checkbox"/>	Pregnant Participants
<input type="checkbox"/>	International research	<input type="checkbox"/>	Employees of a profit or non-profit organization

3. Federal regulations have established guidelines for the equitable selection of participants. Are participants an appropriate group to bear the burdens of this research?

☒ Yes ☐ No      If no, please explain:

Are participants a subset of the population most likely to receive the benefits of this research?

☒ Yes ☐ No      If no, please explain:

**4. Explain any inclusion and exclusion criteria for the study:**

**Inclusion:** English speaking male cyclists and triathletes 18-30 years old with at least one year of competitive experience and currently training at least three times a week for the past eight weeks. The rationale behind using trained athletes is to standardize the physiological response to hypoxia since trained athletes are more impaired than untrained. Also, by using trained athletes, the familiarization effect on the exercise challenges can be limited.

Additionally, nitrate processing is effected by age; therefore, by limiting the age range to 18-30 of the exercise challenge, the variance and reduction seen with older age can be minimized. Athletes must have lived at an elevation of 4000 ft (1300 m) or below for six months with occasional travel to higher altitudes to standardize the adaptations to altitude.

**Exclusion:** Females will be excluded since nitrate processing may be influenced by hormones which can vary with menstruation cycles. Participants will undergo aerobic exercise tests; therefore, participants that smoke, have a history of pulmonary hypertension, high altitude pulmonary edema, cardiovascular disease, cardiopulmonary disease, or physical limitations or injuries will not be allowed to participate. In addition, subjects must not be using supplements or other medications such as aspirin, ibuprofen, and acetaminophen that affect vascular measures.

**5. Recruitment Procedures (how will you find participants?)**

<input type="checkbox"/>	Student Subject Pool; indicate pool: Appalachian State University Club cycling team and local cycling and triathlete clubs
<input type="checkbox"/>	Email/Mailing/Handout
<input checked="" type="checkbox"/>	Website ad/Newspaper ads/Flyers/Postings
<input type="checkbox"/>	School children with request sent to parents
<input checked="" type="checkbox"/>	Participants will be approached by staff members
<input checked="" type="checkbox"/>	Other (explained below)

*A copy of any recruitment materials must be submitted with this application.*

**6. Explain details of recruitment (e.g., obtain list of student emails from Registrar's office and send them recruitment email):** Participants will be approached by staff members at meetings such as club cycling meetings. Subjects will be recruited by word of mouth from

the ASU cycling team and local cycling community. Flyers will be posted around fitness facilities.

7. Does the research include any compensation, monetary inducements, or reimbursement for participation in this research study?

<input type="checkbox"/>	No	<input checked="" type="checkbox"/>	Yes	If yes, explain payment schedule: All subjects will receive \$50.00 upon successful completion of all study requirements.
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### Section V: Informed Consent Process

1. Explain how informed consent will be obtained. *If applicable, include information about: the setting, whether participants will have an opportunity to ask questions, and the roles of any non-research personnel involved. If potential participants or their legally authorized representatives (e.g., parents) are non-English speaking, please explain how the investigator will identify these participants and ensure their ability to understand information about the study to provide consent.*

Subjects will be required to read and sign an informed consent form. This will be obtained in a laboratory setting. Only English speaking participants between 18 and 30 years old will be used. Investigators will be present to describe the study and answer questions. Additional screening processes will be used to ensure participants fit the criteria in section 4.4. A medical history questionnaire, along with diet log and training history will be used. Because of these requirements, only English speaking/reading participants will be permitted into the study. All participants will be legally considered adults and will be of sound mind so they can enter into the contractual agreement without the necessity of a guardian. Participants will be allowed to ask questions at any time.

7. If applicable, describe the safeguards in place to protect the rights and welfare of any vulnerable participants (*e.g., children, prisoners, pregnant persons, or any population that may be relatively or absolutely incapable of protecting their interests through the informed consent process*).

No children, prisoners, or pregnant persons will be recruited. Furthermore, participant's information will not be made available to anyone except the primary and co-investigators.

All information will be under lock and key. Once entered into the study, subjects will be issued a reference number which will be used to blind all personnel except the investigators.

3. Select factors that might interfere with informed consent:

- |                                            |                                                                                                      |
|--------------------------------------------|------------------------------------------------------------------------------------------------------|
| <input checked="checked" type="checkbox"/> | None known                                                                                           |
| <input type="checkbox"/>                   | Research will involve current students in a course/program taught by member of research team         |
| <input type="checkbox"/>                   | Participants are employees whose supervisor is recruiting/requiring participation                    |
| <input type="checkbox"/>                   | Participants have a close relationship to research team                                              |
| <input type="checkbox"/>                   | Other (please specify/indicate any relationship that exists between research team and participants): |

For selected factors, describe any efforts to mitigate:

4. Will participants sign a consent form?

☒ Yes ☐ No

If no, participants must still be provided with a statement regarding the research and one of the following criteria must be met and selected and followed:

- ☐ The only record linking the participant and the research is the consent document and the principal risk is potential harm resulting from a breach of confidentiality, and the research is not FDA-regulated. Each participant will be asked whether he/she wants documentation linking the participant with the research and the participants wishes will govern; OR
- ☐ The research presents no more than minimal risk of harm and involves no procedures for which written consent is normally required outside of the research context.



5. Are you requesting a modification to the required elements for informed consent for participants or legally authorized representatives?

☒ No ☐ Yes If yes, address [criteria to waive elements of consent](#):

## Section VI: Study Procedures

1. Projected data collection dates: Fall 2012- Early Spring 2013

2. Describe research procedures as they relate to the use of human participants. *Information should include what participants will be asked to do, duration of procedures, and frequency of procedures.*

Following procurement of informed consent, subjects will complete a health history questionnaire and an ACSM/AHA Screening Questionnaire designed to prescreen subjects for cardiovascular, respiratory, and metabolic disease. Blood pressure then will be assessed using an arm cuff and stethoscope. Participants will not be allowed to use aspirin, ibuprofen, or compounds containing acetaminophen during the study. No subjects will have previously used or will be currently using dietary supplements in the past month for fat soluble supplements or past week for water soluble supplements. If subjects are found fit to participate, they will come to the laboratory on 3 separate occasions each separated by one week starting one week from the initial visit to perform 3 performance exercise tests. Each visit will be on the same day of the week and same time of day. During this same initial visit a training history will be completed to examine past training status and current training intensity, volume, and level of competition. Height and weight of each subject will be measured before he begins the exercise tests.

On the same day as the screening, participants will perform both a  $\text{VO}_2$  maximum test to exhaustion and a practice time trial of the performance test (~ 200 kJ practice time trial) under a protocol used in a previous study (Morris et al., 2011). All tests and the practice trial will be performed using an electronically braked cycle ergometer (Lode Excalibur Sport Cycle Ergometer, Groningen, Holland) adjusted to the dimensions of the subjects own bicycle, while breathing the hypoxic gas. Following a standardized warm-up, subjects will rest for five minutes while equipment is prepared for the graded exercise test. During the test, each stage will be one minute long (Morris et al., 2011). The power output for the first stage will be set at:  $W=3 \times \text{mass (kg)}$ , and each subsequent stage will increase by  $0.3 W \times \text{mass (kg)}$  (Morris et al., 2011). Expired respiratory gases will be collected continuously, and the gas concentrations will be analyzed by the metabolic cart (Parvomedics Truemax 2400, Consentius Technologies, Sandy, UT). Oxygen consumption, respiratory exchange ratio (RER), and heart rate will be monitored in minute intervals throughout the test. Maximum

oxygen consumption ( $\text{VO}_2 \text{ max}$ ) will be determined based on these variables. Subjects will be allowed to cool down with a comfortable load specified by the subject and to walk around while the equipment is prepared for the practice trial.

A warm up will not be necessary before this practice performance trial. The practice performance trial will be completed breathing hypoxic gas. The Lode cycle ergometer will be set up to allow manual work rate changes. Subjects will be encouraged to complete the trial as fast as they can. The total work for the time trial will be based on each subject's body; 3 kJ / kg of body mass. The only feedback that the cyclist will receive is the work performed displayed by the Lode ergometer. After completion of the prescribed work load, subjects will be allowed to cool down with a comfortable load specified by the subject.

The guidelines of participation will be explained in the informed consent. All subjects will be requested to maintain a consistent diet, training program, and physical activity schedule throughout the experiment. In addition, they will be instructed to consume similar meals and perform a similar degree of physical activity the day before and the day of the race for all three experimental trials. They will be provided a list of foods which they will be required to avoid consuming high levels of nitrates and antioxidants. Subjects will be asked to abstain from caffeine and alcohol twenty-four hours, prior to each performance test. They will be required to keep a log of all fluids and food consumed along with all physical activity performed and quality and quantity of sleep obtained twenty-four hours before each visit. They will be instructed to avoid strenuous physical activity (no more than thirty minutes of greater than or equal to 60% maximum heart rate) twenty-four hours before each performance trial. Participants will be told to drink one liter of fluids the night before and one liter of fluids the morning of each visit. Subjects will undergo a four hour fast in which they will only be allowed to consume water before arriving at the lab. They will be provided with the same high complex carbohydrate, low fat and fiber snack, such as a Cliff bar, to eat two hours before they arrive at the lab. Once at the lab, they will not be allowed to eat or drink (other than the treatment fluid) three hours before the performance trial. Furthermore, they will not be allowed to drink or consume any fluids or food after the treatment is ingested until after the performance test is complete. Subjects will be asked to abstain from caffeine and alcohol twenty-four hours prior to each race. Finally, subjects will not be allowed to use chewing gum or anti-bacterial mouthwash while involved in the study since these are known to destroy the oral bacteria necessary for the conversion of nitrate to nitrite (Govoni et al., 2008).

Participants will arrive at the Vascular, Biological, and Autonomic Studies Laboratory at Appalachian State University, room 186C, University Hall, three hours before each performance test and will be asked to sit down and rest quietly for fifteen minutes. Fifteen minutes after arrival, systemic blood pressure will be measured using an arm stethoscope and cuff. Thirty minutes after arrival, subjects will be required to drink the treatment. The supplemental fluid will contain either 91 ml of organic beetroot juice (BR) (containing ~5.77 mMol of nitrate; Beet it, James White Drinks Ltd., Ipswich, UK), 91 ml of organic nitrate depleted beetroot juice (PL) similar to the beetroot juice in color, taste, texture, and odor (containing ~0.048 mMol of nitrate; Beet it, James White Drinks Ltd., Ipswich, UK)

(Lansley et al. (2011)), or 91 ml of water (CO). The treatment will be assigned and administered in a randomized double blind design.

Two hours and forty-five minutes after arrival, resting systemic blood pressure will be measured. A standardized 10 minute warm-up will be performed under hypoxic conditions prior to commencement of each performance test. Immediately following the warm-up, another systemic blood pressure measurement will be taken. Subjects will then rest for five minutes while the equipment is prepared for the performance test. The Lode ergometer will be placed in time trial mode to allow for adjustment of work rates. Fifteen minutes past the warm-up start time, subjects begin the performance test. Subjects will be able to change work rates as desired throughout the test with the objective of completing the prescribed work load as fast as possible. As in the practice trial, cyclists will receive feedback only on their work completed and will be blinded to elapsed time and power output. The investigators will give verbal encouragement similar to that in the pre-supplementation trial.  $\text{VO}_2$  and RER will be monitored with the metabolic cart throughout the performance test. Blood oxygen saturation, elapsed time, and heart rate will be recorded at 0.25, 0.50, 0.75, and 1.00 of the total kilojoules completed. Blood oxygen saturation and heart rate will be measured using a pulse oximeter that will be clipped to the subject's earlobe. After completion of the trial, subjects will be allowed to remove the mouth piece and nose clip and cool down.

During all of the pre-supplementation tests and experimental tests, each subject will be exposed to hypoxic gas similar to the set up used in a previous study (Morris et al., 2000). The device used was designed and tested by Dr. Morris. He has used this device to manipulate inspired oxygen concentrations in several research studies and in hundreds of training sessions using athletes at the United States Olympic Training Center in Colorado Springs, CO and in a previous investigation at Appalachian State University (Study# 11-0065 Effects of garlic on exercising blood oxygen desaturation and performance in hypoxia). The device consists of a cylinder containing medical grade breathing gas of 6% oxygen 94% nitrogen mixture. This mixture is pumped into meteorological balloon which serves as a reservoir. Leading from the balloon is a breathing tube that is attached to a dual intake valve. The dual intake valve has three ports: Two of these ports allow for intake of air with one of these ports leading to the atmosphere and the other leading to a gas reservoir containing the hypoxic gas. The third port on the dual intake valve leads to a hose that is attached to the inspired port of a standard Hans Rudolph model 2700 breathing valve. The subject is attached to the breathing port of this Hans Rudolph valve. As the subject inspires, equal amounts of air will flow from the gas reservoir and the atmosphere through the dual intake valve ports. The resultant mixture delivered to the subject will have an oxygen concentration of ~13% which provides a similar  $\text{PIO}_2$  to that experienced at an altitude of 14,000 ft. The use of the dual intake valve provides two advantages: 1. Should any part of the hypoxic gas reservoir system become plugged, or should the gas cylinder/reservoir become empty while a subject is using the device, air delivery will be maintained through the atmospheric port of the dual intake valve. 2. Mixing the dry gas mixture from the cylinder with atmospheric air humidifies the air and helps prevent the subject from developing a dry mouth and throat which can lead to excess mucous production.

The total involvement time will be approximately 14 hours over 30 days. The initial visit will last approximately 90 minutes and the final 3 visit will require four hours of time. Both the VO2 max and performance tests will be preceded by a 10 minute warm-up under hypoxic conditions. Previous research by the author suggests that the VO2 max test will last 5-7 minutes and the performance trial will take 15-20 minutes under hypoxia...

## Experimental Trial ~200 kJ Time Trial

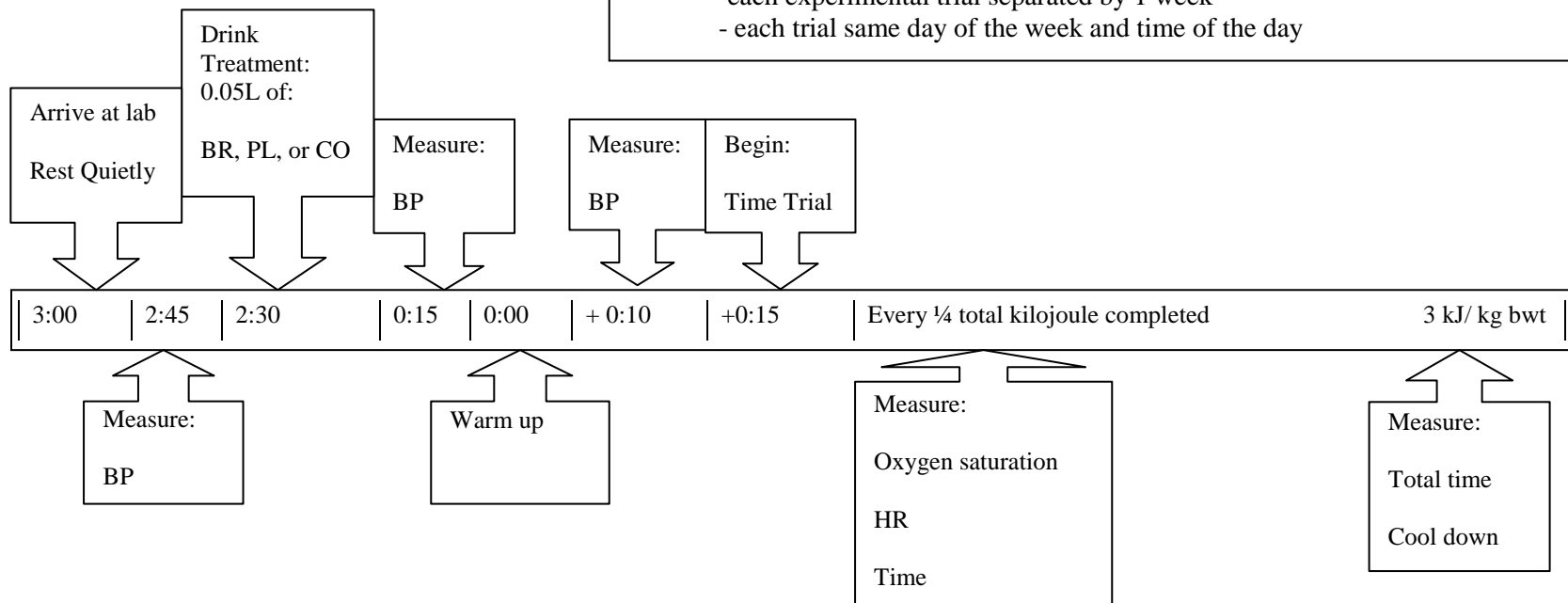
### Treatment Fluids:

Acute (1 dose) supplementation

- 91 ml Organic beetroot juice (BR) (~5.77 mMol nitrate)
- 91 ml Nitrate depleted organic beetroot juice (PL) (~0.048 mMol nitrate)
- 91 ml water

\*Crossover design: Each subject will experience each treatment.  
-randomly assigned order  
-each experimental trial separated by 1 week  
- each trial same day of the week and time of the day

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**3. Participants' identification (check one):**

- ☐ Information is collected so that participants CANNOT be identified directly (by names, images or other identifiers) or indirectly (by linking responses to participants).
- ☒ Information is collected so that participants CAN be identified, either directly or indirectly, by the research team but identifying information will not be disclosed publicly.
- ☐ Information is collected so that participants CAN be identified, either directly or indirectly, by the research team and identifying information will be disclosed publicly.

**4. Check all locations of study procedures that apply:**

- ☐ N/A – online survey
- ☒ Appalachian campus, indicate building: Vascular Biology and Autonomic Studies Laboratory, Institute of Health and Human Services, Boone, NC
- ☐ School system(s):
- ☐ Human Performance Lab, NCRC
- ☐ Off-campus location(s). List:

**5. Data collection**

**5a. Please check all data collection activities involved in this study:**

- ☒ Paper Surveys / Questionnaires
- ☐ Online Surveys / Questionnaires      Name of Survey Provider:
- ☐ Telephone Surveys / Questionnaires      Name of Survey Provider:
- ☐ Standardized Written / Oral / Visual Tests
- ☒ Interviews
- ☐ Focus Groups
- ☐ Tasks
- ☐ Public Observation
- ☐ Classroom Observation/Work Site Observation

- ☐ Voice, video, digital or image recordings made for research purposes
- ☐ Materials (i.e., data, documents, records/specimens) that have been collected or will be collected for **non research** purposes
- ☒ Collection or study of materials (i.e., data, documents, records/specimens) that are publicly available or if the information is recorded so that participants cannot be identified, directly or indirectly through identifiers
- ☐ Materials (i.e., data, documents, records/specimens) that have been collected for another research project
- ☒ Moderate exercise and muscular strength testing
- ☒ Other: Maximal Exercise

**5b.** If your study does not involve biomedical procedures skip to question #6. Otherwise, select all data collection activities that apply:

- ☐ Blood samples by finger stick, heel stick, ear stick or venipuncture

Indicate the type of participants and how much blood will be drawn:

- ☐ from healthy, non pregnant adults who weigh at least 110 pounds
- ☐ from other adults or children
- ☐ How many times per week will blood be drawn?
- ☐ How much blood will be drawn at one time?
- ☐ How much blood will be drawn in an 8-week period?
- ☐ How often will collection occur?

- ☐ Noninvasive procedures to collect biological specimens for research purposes
- ☐ Sterile Surgical/Invasive procedures
- ☐ Banking of biological materials
- ☒ Noninvasive procedures to collect data such as use of physical sensors applied to surface of body and electrocardiography

- ☐ Procedures involving x-rays (e.g., DEXA scan for body composition)

<input type="checkbox"/>	Ingestion of wholesome foods without additives
<input checked="" type="checkbox"/>	Ingestion/application of substances other than wholesome foods without additives
<input type="checkbox"/>	Clinical study of a drug/medical device
<input type="checkbox"/>	Obtaining medical data from a health care provider, health plan or health care clearinghouse
<input type="checkbox"/>	Genetic Testing
<input type="checkbox"/>	Other:

**5c.** Is this research FDA-regulated (i.e., It is an experiment that involves one or more of the following test articles: foods/dietary supplements that bear a nutrient content/health claim, infant formulas, food/color additives, drugs/medical devices/biological products for human use)?

☒ No ☐ Yes

**6.** Is deception involved?

☐ No ☒ Yes If yes, please describe: Subjects will be blinded to the application of the treatments

**7.** Does the data to be collected relate to any illegal activities (e.g., immigration status, drug use, abuse, assault)?

☒ No ☐ Yes If yes, please describe:



## Section VII: Confidentiality and Safeguards

1. In most cases, the research plan should include adequate provisions to protect the privacy of subjects. How will the confidentiality of participants be maintained (e.g., how will access to participants be controlled)?

The individual's file and hardcopy data will be locked in a file cabinet within a locked office in a secure building.

2. Will collected data be monitored to ensure the safety of subjects (e.g., survey includes a question about suicidality so the investigator will...)?

<input type="checkbox"/>	No	<input checked="" type="checkbox"/>	Yes	If yes, please explain procedures to ensure safety of participants: Data written in reports and manuscripts will not be linked to or identify individual subjects. Subjects will further be protected from being identified by avoiding photography and the use of personal names. No harmful use of the data is intended. If any issues arise the PI will be notified and will address each situation. Participants will be regularly asked about their willingness to continue their participation along with their compliance to the protocol throughout each visit.
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3. Describe what will be done with the data and resulting analysis:

The data will be coded and entered into a computer spreadsheet and all individual identifiers will be removed and replaced with a subject number. Following that, analysis will be run and results will be published in peer-reviewed journals. All hardcopy data will be destroyed and the computer files will be erased (destroyed) 5 years following the protocol. All data will be used only for the purpose of this study and no data will be shared with anyone outside the immediate study team.

4. Describe measures you are taking to safeguard study data (check all that apply):

<input checked="" type="checkbox"/>	Data is not linked to identifying information
<input checked="" type="checkbox"/>	Maintain consent forms in a separate location from data
<input checked="" type="checkbox"/>	Using subject codes on <u>all</u> collected data and maintaining the key linking subject codes with

identifiable information in a separate location from data

\_\_\_\_\_

<input checked="" type="checkbox"/>	Locking cabinets/doors. List location: 053 Holmes Convocation Center
<input checked="" type="checkbox"/>	Data kept in area with limited public access. List location: 053 Holmes Convocation Center
<input checked="" type="checkbox"/>	Password protected computers
<input type="checkbox"/>	Encryption
<input type="checkbox"/>	PDA's and removable media (e.g., CDs, etc.) will be kept in a secure location. List location:
<input type="checkbox"/>	Other, please describe:

## 5. Data Sharing

**5a.** What type of data will be shared? (*Note: Sharing includes releasing, transmitting and providing access to outside of the research team.*) Check all that apply:

<input type="checkbox"/>	Data collected anonymously
<input type="checkbox"/>	Anonymized or De-linked data. Identity was once associated with data/specimen but identifying information destroyed
<input type="checkbox"/>	Coded and linked data (Data is coded. With the code, the data may be linked back to identifiers, but the link back to identifiers will not be shared.)
<input type="checkbox"/>	Identifiable Data (e.g., names, email addresses, date of birth, IP addresses)

Indicate which secure method(s) of transmission will be used:

**5b.** If identifiable data will be shared within or outside of the research team, please explain how it will be shared (check all that apply):

<input type="checkbox"/>	Secured Website. Please provide name of website:
<input type="checkbox"/>	Encrypted email
<input type="checkbox"/>	U.S. Postal Service or other trackable courier services
<input type="checkbox"/>	Fax in a secured area
<input checked="" type="checkbox"/>	Shared drive with password protection
<input type="checkbox"/>	Personal delivery by member of research team

<input type="checkbox"/>	Private telephone conversation to member of research team
<input type="checkbox"/>	Other, please describe:

**6. Secure Disposal:** *Note: consent forms should be stored for 3 years after study completion.*

**6a.** How long will the data be stored?

<input type="checkbox"/>	1 year after study conclusion	<input checked="" type="checkbox"/>	5 years after study conclusion
<input type="checkbox"/>	Indefinitely	<input type="checkbox"/>	Data without identifiers stored indefinitely
<input type="checkbox"/>	Other, please describe (e.g., sponsor requirements):		

**6b.** How will data be destroyed?

<input checked="" type="checkbox"/>	Paper will be shredded	<input type="checkbox"/>	Biological samples will be destroyed by:
<input checked="" type="checkbox"/>	Destroy electronic files from computer/PDAs/removal media (CDs, diskettes) by: deletion		
<input type="checkbox"/>	Other, please describe:		

## Section VIII: Risk and Benefits of Study

**1.** The risks to participants must be reasonable in relation to anticipated benefits, if any, to participants and the importance of the knowledge that may be reasonably be expected to result. Select all applicable:

<input checked="" type="checkbox"/>	Participants of the study may directly benefit by (describe): <i>[Note that compensation is not considered a benefit.]</i>
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Beetroot juice may help improve aerobic exercise performance at high altitude. Some cycling and triathlete competitions are held at moderate to high altitude where aerobic performance is impaired. All subjects will gain awareness of their performance results and physiological responses in relation to simulated high altitude. They may also gain a better understanding of methods to alleviate hypoxia induced pulmonary hypertension and improvement of exercise performance in high-altitude environments.

☒

Society may benefit from the study by (describe):

This treatment could potentially help athletes improve exercise performance at high altitude. Evidence of this mechanism could be very beneficial to multiple populations. Not only could it help non-acclimatized athletes be more competitive at moderate to high altitude events, but it could also help military personnel and other workers who perform duties at high altitude or hypoxic environments. In addition, dietary nitrate induced pulmonary vasodilation may be a prospective aid for reducing pulmonary hypertension, hypoxia, and pulmonary edema, ultimately improving everyday performance in diseased populations (Zuckerbraun et al., 2011).

**2. Describe the potential risks (e.g., psychological, legal, physical, social harm, loss of confidentiality) to any individual participating in this project:**

Possible side effects for moderate to maximal-intensity cycling include brief feelings of nausea, lightheadedness, or dizziness after the completion of the exercise, especially when exercise is performed in hypoxia. Exercise testing with gradually increasing workloads to the point of fatigue is associated with a very minimal risk of death (0.01%) and complications of the heart (0.04%). Exercise in hypoxia increases the likelihood of lightheadedness and/or dizziness. However, breathing of hypoxic mixtures during rest and exercise is a relatively safe procedure that has been in use in research settings for many years. The gas mixture we are proposing simulates an altitude of 14,000 feet. This level of hypoxia has been studied in numerous laboratories in the past without adverse effects. Additionally, the Maher Research Laboratory, located at 14,000 feet on Pike's Peak, routinely studies human subjects during rest and heavy exercise without encountering significant adverse effects. Dr. Morris spent 10 years working as a physiologist for the United States Olympic Committee and USA Cycling. During this period he performed research and oversaw hundreds of training sessions that utilized maximal exercise in hypoxia. While minor symptoms such as dizziness and lightheadedness were occasionally observed, severe symptoms such as fainting were never observed and he has never had to terminate an exercise test or training session due to adverse reactions to hypoxia. The symptoms that do occur while breathing hypoxic air quickly subside when the subject returns to breathing normoxic air.

This is the same protocol that was approved previously to test garlic supplementation on hypoxic aerobic exercise performance.

**3. Assessment of level of risk:**

☒

Risks (including physical, emotional, social, legal or financial) are the same as encountered in daily life or during the performance of routine physical or psychological examinations or tests (minimal risk).

☐

Risks are more than minimal in that either: a) the probability of harm or discomfort anticipated, or b) the magnitude of harm or discomfort anticipated is greater than that

encountered in daily life.

- ☐ Information to be collected could cause participants to be at risk of criminal or civil liability if responses are disclosed outside of the research setting.
- ☐ Information to be collected could be damaging to participant's financial standing, employability, or reputation if disclosed outside of the research setting.

**3. Describe procedures for protecting against, or minimizing, the potential risks:**

All subjects will be highly trained cyclists with no history of adverse cardiac risk. These risks are exceptionally low in this subject group, whose members frequently (several times per week) participate in training sessions or competitions having physical demands similar to the protocols of the proposed study. Typical weekly training volumes for these types of subjects are at least 4-5 hours per week with .5-1 hours per week in excess of 90% of maximal heart rate. At least two technicians trained in CPR will be present for all tests. All research team members will have previous exercise testing experience and will be aware of the signs and symptoms associated with possible adverse reactions during exercise. To further ensure a low risk subject population, each subject will be screened using a Health History Questionnaire and the ACSMAHA Screening Questionnaire. Only subjects that are classified as "low risk", ie no more than 1 risk factor for cardiovascular disease, by ACSM risk stratification rankings will be retained as subjects. During exercise in hypoxia, subjects will be observed closely for significant adverse reactions such as loss of consciousness and exercise will be terminated should any of these symptoms be observed. Blood oxygen saturations will be monitored throughout the exercise and recovery periods. Typical blood oxygen saturation during rest at sea-level are 98%-100%. During maximal exercise these levels will drop into the mid 80% range (Rowell et al, 1964). Numerous investigations have executed maximal exercise tests at simulated altitude that are similar to the altitude that we are proposing (Cymmerman et al, 1989; Favier et al 1995; Hughson et al, 1995; Stoneham et al, 1993) and have observed blood oxygen saturations at maximum exercise averaging in the mid 60% to low 70% range with the lowest observed oxygen saturation being 50% during maximal exercise at a PIO<sub>2</sub> of 88 Torr. Additionally, Cymmerman et al, 1995, studied maximum oxygen consumption in subjects at a simulated altitude of 8800 meters or PIO<sub>2</sub> of 43 Torr (Mt Everest) and observed average blood oxygen saturations of 35% during maximal exercise. None of these investigators observed any serious or long term negative effects to hypoxia and, after removal from hypoxia, blood oxygen saturations typically returned to normal within 1 - 2 minutes. We do not anticipate oxygen saturations below 50% and will immediately halt exercise and return to normoxia any subject who exhibits oxygen saturations below 45%. Upon termination of the VO<sub>2</sub> max and performance tests, subjects will be returned immediately to normoxia and be given 10 minutes of low intensity cycling followed by 5 minutes of rest in the seated position. During these periods, the subject's blood oxygen saturations will be monitored continuously for any negative reactions to exercise and/or hypoxia.

5. If human subject data/specimens will be used for future research that is not described above, please explain. (Future use of data/specimens should be disclosed to the participant in the informed consent.) N/A

Please check any materials below that will be submitted with your application. Note: please submit as separate files.

<input checked="" type="checkbox"/>	Recruitment wording
<input checked="" type="checkbox"/>	Consent form(s)
<input type="checkbox"/>	Letter(s) of Agreement
<input type="checkbox"/>	Research Personnel Form(s)
<input checked="" type="checkbox"/>	Instruments (Survey questions, interview questions, etc.)
<input type="checkbox"/>	Copy of grant/contract/agreement
<input checked="" type="checkbox"/>	Other (please describe): Data Forms, Health History Questionnaire, Diet, Physical Activity, and Sleep logs, Diet and Exercise instructions

Please **send an electronic Word attachment (not scanned) of this application and any accompanying materials to [irb@appstate.edu](mailto:irb@appstate.edu)**. Thank you for taking your time to promote ethical human participant research at Appalachian!

#### References:

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## **Appendix B**

### **Informed Consent**

## **Consent to Participate in Research**

### *Information to Consider About this Research*

#### **Beetroot Juice Dietary Nitrate Supplementation and Hypoxic Aerobic Exercise Performance**

Co-Principal Investigator: Dr. David Morris

Department: HLES

Contact Information:

Appalachian State University

53 Holmes Center

111 Rivers St.

Boone, NC 28607

[morrisdm@appstate.edu](mailto:morrisdm@appstate.edu)

828-262-7120

Principle Investigator: Jennifer Arms

[armsjc@appstate.edu](mailto:armsjc@appstate.edu)

### **What is the purpose of this research?**

Exposure to high-altitude environments has been shown to reduce blood oxygen levels and hinder the circulation of blood through the lungs. These responses contribute to reductions in aerobic exercise performance at high-altitude. Nitric oxide is a compound that occurs naturally in the body and helps to increase blood flow. Nitric oxide levels can be increased by consuming foods that contain high levels of nitrates. Beetroot juice contains high levels of nitrates and its consumption has been shown to increase forearm blood flow and improve exercise performance at sea level. However, the ability of beetroot juice consumption to improve blood circulation in the lung, blood oxygen levels, and exercise performance in high altitude environments has not been studied. By doing this research, we hope to learn if a small dose of beetroot juice consumed 2.5-3 hours prior to exercise can increase blood oxygen levels and cycling time trial performance.

### **Why am I being invited to take part in this research?**

You are being invited to take part in this research because you are a healthy, highly-endurance trained, non-smoking male cyclist or triathlete who is accustomed to performing high-intensity exercise.

### **Are there reasons I should not take part in this research?**

If you do not conform to the above listed requirements, or if you have any known cardiovascular, respiratory (pulmonary hypertension, high altitude pulmonary edema) or metabolic diseases, you should not participate in this study. If you volunteer to take part in this study, you will be 1 of about 12 people to do so.

### **What will I be asked to do?**

The research procedures will be conducted at Appalachian State University, Vascular, Biological, and Autonomic Studies Lab, RM 186C, University Hall. You will need to come to this location 4 times during the study. Your initial visit will require about 90 minutes and your final 3 visits will each require about 4 hours of your time. The total amount of time you will be asked to volunteer for this study is approximately 14 hours over the next 30 days.

During your initial visit, you will be asked to complete a health history questionnaire and the American College of Sports Medicine/ American Heart Association screening questionnaire designed to prescreen subjects for cardiovascular, respiratory, and metabolic disease. Blood pressure will then be assessed using an arm cuff and stethoscope. You will also be required to fill out a training history. If you are declared fit to participate, you will then perform a progressive, maximal exercise test to exhaustion so that we can determine your VO<sub>2</sub> max and the work rate that elicits your VO<sub>2</sub> max. VO<sub>2</sub> max is a measure of your aerobic fitness. It allows us to determine how much oxygen your body can use to provide fuel for your working muscle. Immediately prior to starting the VO<sub>2</sub> max test, you will be required to perform a 10-minute warm-up at a low to moderate intensity. Then the max test itself will start at a low to moderate level intensity which will be increased by a small amount each minute until you are exhausted. The maximal test itself generally takes about 6-8 minutes to complete. During the test, a snorkel-like mouthpiece will be placed in your mouth and your nose will be plugged with a nose clip. This will allow us to collect your expired air and analyze it to determine your oxygen consumption level. The maximal test will be performed under a simulated high-altitude condition. To achieve this condition, you will be breathing medical grade gas with an oxygen concentration that is lower than what is found in the atmosphere. This altered gas mixture will simulate an altitude of approximately 14,000 ft. Once you have completed the maximal test, the mouthpiece will be removed and you will be able to breathe normal sea level air. You will then be allowed to recover for approximately 20 minutes. Following your recovery, you will be asked to perform a practice trial of an exercise test that you will be performing on your subsequent visits. This practice trial will consist of time trial of approximately 12 km in length. During this trial, you will be able to adjust the work rate (intensity) of your exercise at your discretion, but you will be encouraged to finish prescribed distance as quickly as possible. This trial will also be performed while breathing the low oxygen gas mixture and will require approximately 20 minutes to complete. Once the practice trial is completed, you will be able to cool down on the bike breathing normal sea level air. All of these tests and the tests that are performed on subsequent visits will be performed on a bicycle ergometer with dimensions that will match your own bicycle.

Following the initial evaluation, you will be asked to return to the lab on 3 occasions, each visit will be separated by a week. You will be placed on the following exercise and diet restrictions during your involvement: avoid strenuous exercise and maintain a consistent diet on the day prior to each visit, avoid alcohol and caffeine consumption for 24 hours prior to each visit, avoid anti-bacterial mouth wash and chewing gum while participating in the study, and avoid the foods on the list that will be given to you of high nitrate and antioxidant containing foods throughout the study. You will be required to keep a log of all fluids and food consumed as well as all physical activity completed and quality and quantity of sleep 24 hours prior to each trial. You will be asked to drink at least one liter of fluids the night before and morning of the trial. You will be given a small snack (2 Clif Bars) to be consumed two hours before your arrival at the laboratory, but will otherwise be required to avoid consuming food (other than water) for 4 hours prior to each visit. Upon arrival at the laboratory, you will be seated and will rest quietly for 10-15 minutes, after which blood pressure will be taken. Thirty minutes after arrival, you will be then be given 1 of 3 supplement options:

91ml of beetroot juice, 91ml of a placebo juice, or 91ml of water. You will then be asked to sit quietly for 135 minutes. During this period you will not be allowed to perform physical exercise, but you will be allowed to perform sedentary activities such as reading, studying, watching videos or listening to music. We will then take another blood pressure measurement with a stethoscope and blood pressure cuff. You will then begin a 10-minute warm-up while breathing the low oxygen gas mixture. Following the warm-up you will be allowed approximately 5 minutes to stretch and use the restroom. You will then remount the bicycle ergometer, begin breathing the low oxygen gas mixture and complete the 12 km time trial. During each time trial you will have a sensor that looks like a small ear clip, clipped to your ear lobe to monitor blood oxygen saturation and heart rate. You will be allowed to adjust your work rate during the time trial as you desire, but you will be encouraged to finish each trial as quickly as possible. You will be blinded to your actual work rate, but the cumulative distance completed will be constantly updated and displayed by a digital readout. Upon completion of the trial, you will take out the mouth piece and take off the nose clip and will be allowed to cool down.

# Experimental Trial ~200 kJ Time Trial

## Treatment Fluids:

Acute (1 dose) supplementation

- 91 ml Organic beetroot juice (BR) (~5.77 mMol nitrate)
- 91 ml Nitrate depleted organic beetroot juice (PL) (~0.048 mMol nitrate)
- 91 ml water

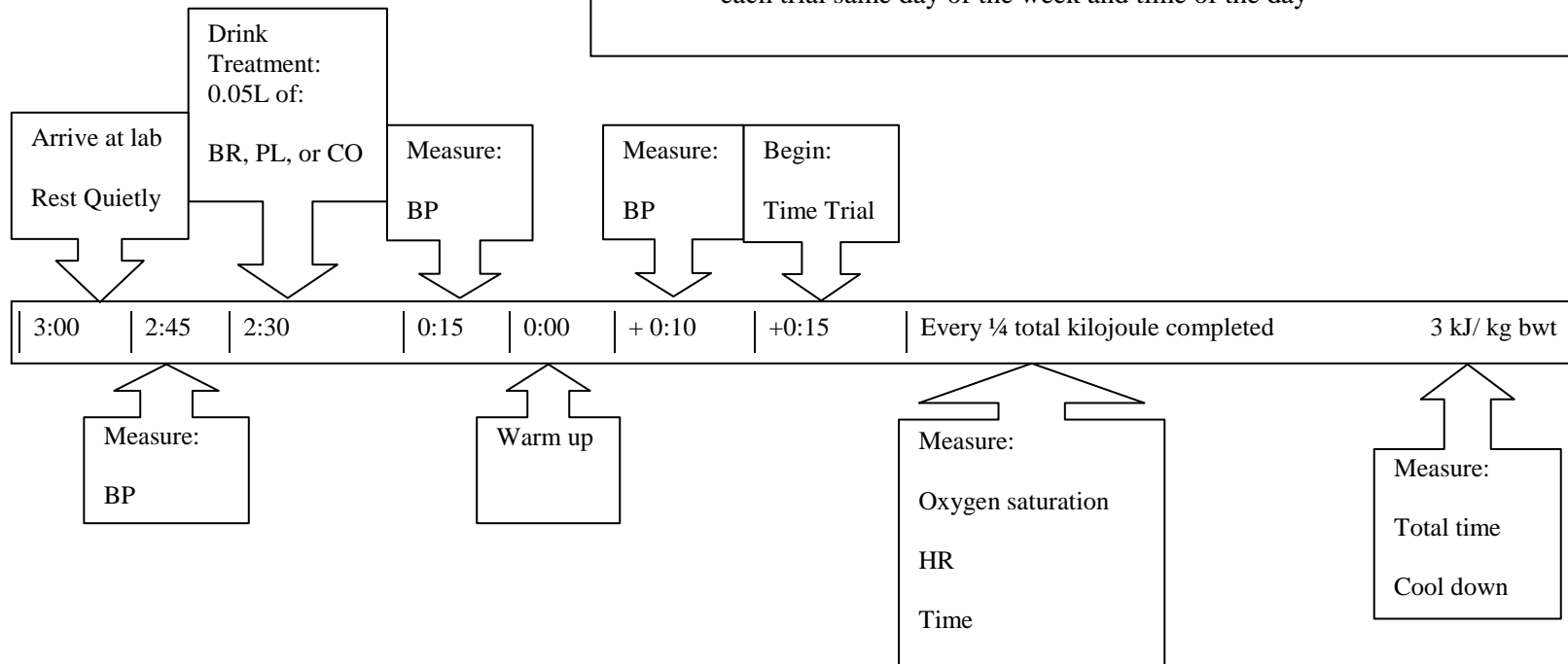
\*Crossover design: Each subject will experience each treatment.

-randomly assigned order

-each experimental trial separated by 1 week

- each trial same day of the week and time of the day

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## **What are possible harms or discomforts that I might experience during the research?**

We know about the following risks or discomforts that you may experience if you choose to volunteer for this study:

Possible side effects for moderate to maximal-intensity cycling include brief feelings of nausea, lightheadedness, or dizziness after the completion of the exercise, especially when exercise is performed in hypoxia. Exercise testing with gradually increasing workloads to the point of fatigue is associated with a very minimal risk of death (0.01%) and complications of the heart (0.04%). Exercise in hypoxia increases the likelihood of lightheadedness and/or dizziness. However, breathing of hypoxic mixtures during rest and exercise is a relatively safe procedure that has been in used in research settings for many years.

Nitrate supplementation via beetroot juice consumption has been shown to cause dilation of the blood vessels in the forearm. Therefore, nitrate supplementation may potentially decrease blood pressure. However, nitrate supplementation has not been shown to reduce blood pressure to unsafe levels which would produce symptoms such as light headedness, dizziness, or feeling faint.

Some research using animal models suggests the body can convert nitrates into substances that are potentially harmful. However, direct evidence of nitrate related cancer from long term dietary nitrate consumption is inconclusive and lacking in human studies. Research also suggests that this conversion to carcinogenic compounds from dietary nitrate ingestion may not be the favored pathway in children and adult humans. Not all nitrate sources have the same effect and recent evidence suggests that substances found in fruits and vegetables such as Vitamin C may reduce the formation of these harmful substances. It has been shown that people who have a high nitrate diet through a high consumption of fruits and vegetables have a lower incidence of mortality due to gastric cancer. The World Health Organization has set a daily acceptable intake of 0- 3.7 mg/ day. Americans typically consume 40-100 mg of nitrate per day through their diet with the highest sources of nitrate being celery, lettuce, spinach, and beets. A 2-inch beet that weighs about 82 grams (250 mg of nitrate per 100 grams) contains about 205 mg of nitrate. We would be asking you to undergo a short term supplementation. You would consume approximately 357.5 mg one time for the beetroot juice treatment (BR) and approximately 0.2 mg of nitrate one time for the placebo intervention throughout the entire study.

### **Are there any reasons you might take me out of the research?**

If you do not follow the protocol, you will not be allowed to continue to participate in the study. During the study, information about this research may become available that would be important to you and may affect your willingness to participate. This might include information about the side effects that are caused by taking part in this study. If this happens, we will tell you about these new side effects and let you decide whether you want to continue to take part in the research.

There may be reasons we will need to remove you from the study, even if you wish to continue to participate. We may find out that it is not safe for you to stay in the study. It may be that the side effects from the treatments or hypoxic exercise are so severe that we need to stop the study or take you out of the study to reduce your risk of harm. If we find that the research might harm you or that it is not providing enough of a benefit to justify the risks you are taking, we will terminate your involvement in the study. We may also find that you are not or cannot take your supplement properly or you are not or cannot come for your study visits as scheduled. If these things are found to be true, we will need to take you out of the study.

### **What are possible benefits of this research?**

You will learn your VO<sub>2</sub> max and time trial performance results that would be applicable if you were competing at high altitude. This research should help us learn more about dietary nitrate supplementation and if it can improve aerobic exercise performance at hypoxia/ high altitude. The information gained by doing this research may help you and others to compete in hypoxic/ high altitude environments.

### **Will I be paid for taking part in the research?**

We will pay you \$50.00 for the time you volunteer while being in the study. You must complete the entire protocol to receive your payment. You will need to provide your address when you complete the form for payment.



### **How will you keep my private information confidential?**

Your information will be combined with information from other people taking part in the study. We intend to publish the results of the study so that it may be shared with other researchers. This publication will only contain the combined information from you and the others who participated in the study. You will not be personally identified in any published or presented materials.

All personal information obtained during the course of your participation will be accessible only to Dr. Morris and members of his research team. You will be identified by code to preserve your confidentiality and your file will be kept in a locked cabinet in Dr. Morris's office. We will make every effort to prevent anyone who is not part of the research team from knowing that you gave us information or the content of that information. However, there are some circumstances in which we may have to show your information to other people. For instance, we may be required to show information that identifies you to people who need to be sure that we have done the research correctly, such as Appalachian State University's Institutional Review Board. Data from this research will be kept indefinitely, however, codes linking you to your data will be destroyed three years following the completion of the data collection.

### **What if I get sick or hurt while participating in this research study?**

If you need emergency care while you are at the research site, it will be provided to you. If you believe you have been hurt or if you get sick because of something that is done during the study, you should call your doctor or, if it is an emergency, call 911 for help. In this case, tell the doctors, the hospital or emergency room staff that you are taking part in a research study and the name of the Principal Investigator. If possible, take a copy of this consent form with you when you go. Call the Co-Principal Investigator, David Morris, at 828-262-7120 as soon as you can. He needs to know that you are hurt or ill.

There are procedures in place to help attend to your injuries or provide care for you. Costs associated with this care will be billed in the ordinary manner to you or your insurance company. However, some insurance companies will not pay bills that are related to research costs. You should check with your insurance company about this. Medical costs that result from research-related harm may also not qualify for payments through Medicare, or Medicaid. You should talk to the Principal Investigator about this, if you have concerns.

### **Who can I contact if I have a question?**

The people conducting this study will be available to answer any questions concerning this research, now or in the future. You may contact the Co-Principal Investigator, David Morris at 828-262-7120. If you have questions about your rights as someone taking part in research, contact the Appalachian State University Institution Review Board Administrator at 828-262-2130 (days), through email at [irb@appstate.edu](mailto:irb@appstate.edu) or at Appalachian State University, Office of Research and Sponsored Programs, IRB Administrator, Boone, NC 28608.

### **Do I have to participate? What else should I know?**

Your participation in this research is completely voluntary. If you choose not to volunteer, there will be no penalty and you will not lose any benefits or rights you would normally have. If you decide to take part in the study you still have the right to decide at any time that you no longer want to continue. There will be no penalty and no loss of benefits or rights if you decide at any time to stop participating in the study.

This research project has been approved by the Institutional Review Board (IRB) at Appalachian State University. This study was approved on 6/1/2012. This approval will expire on 4/18/2013 unless the IRB renews the approval of this research.

### **I have decided I want to take part in this research. What should I do now?**

The person obtaining informed consent will ask you to read the following and if you agree, you should sign this form:

- I have read (or had read to me) all of the above information.
- I have had an opportunity to ask questions about things in this research I did not understand and have received satisfactory answers.
- I understand that I can stop taking part in this study at any time.
- By signing this informed consent form, I am not giving up any of my rights.
- I have been given a copy of this consent document, and it is mine to keep.

---

Participant's Name (PRINT)  
Date

Signature

## **Appendix C**

Questionnaires, Study Requirement Instructions, and Reporting Logs

## ASU/NCRC SCREENING QUESTIONNAIRE\*

ID NUMBER \_\_\_\_\_

**Assess your health status by marking all true statements**

### MEDICAL HISTORY

- ☐ a heart attack
- ☐ heart surgery
- ☐ cardiac catheterization
- ☐ coronary angioplasty (PTCA)
- ☐ pacemaker/implantable cardiac
- ☐ defibrillator/rhythm disturbance
- ☐ heart valve disease
- ☐ heart failure
- ☐ heart transplantation
- ☐ congenital heart disease

**SYMPTOMS**

\_\_\_\_\_ You experience chest discomfort with exertion.

\_\_\_\_\_ You experience unreasonable breathlessness.

\_\_\_\_\_ You experience dizziness, fainting, or blackouts.

\_\_\_\_\_ You take heart medications.

**OTHER HEALTH ISSUES**

\_\_\_\_\_ You have diabetes.

\_\_\_\_\_ You have asthma or other lung disease.

\_\_\_\_\_ You have burning or cramping sensation in your lower legs when walking.

\_\_\_\_\_ You have musculoskeletal problems that limit your physical activity.

\_\_\_\_\_ You have concerns about the safety of exercise.

\_\_\_\_\_ You take prescription medication(s).

\_\_\_\_\_ You are pregnant.

*If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a medically qualified staff.*

**CARDIOVASCULAR RISK FACTORS**

\_\_\_\_\_ You are a man older than 45 years.

\_\_\_\_\_ You are a woman older than 55 yrs, had a hysterectomy, or are postmenopausal.

\_\_\_\_\_ You smoke, or quit smoking within the previous 6 months.

\_\_\_\_\_ Your blood pressure is >140/90 mm Hg, or take medication.

\_\_\_\_\_ Your blood cholesterol level is >200 mg/dL (borderline or high), or take meds.

- \_\_\_\_\_ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister).
- \_\_\_\_\_ You are physically inactive (i.e., you get <30 minutes of physical activity on at least 3 days per week).
- \_\_\_\_\_ Your body mass index is 30 or higher.

*If you did not mark any of these or just one, you should be able to exercise safely without consulting an MD in a self-guided program or almost any facility that meets your exercise program needs. If you marked two or more of the statements in this section you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a professionally qualified exercise staff to guide your exercise program.*

*\*2010 AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire*

### **2010 ACSM Risk Stratification**

**Low Risk – individuals classified as Low Risk** are those who do not have signs/symptoms of or have diagnosed cardiovascular, pulmonary, and/or metabolic disease, and have no more than one (i.e.,  $\leq 1$ ) CVD risk factor. The risk of an acute cardiovascular event in this population is low and a physical activity/exercise program may be pursued safely without the necessity for medical examination and clearance.

**Moderate Risk – individuals classified as Moderate Risk** do not have signs/symptoms of or diagnosed cardiovascular, pulmonary, and/or metabolic disease, but have two or more (i.e.,  $\geq 2$ ) risk CVD factors. The risk of an acute cardiovascular event in this population is increased although in most cases individuals at moderate risk may safely engage in low to moderate intensity physical activities without the necessity for medical examination and clearance. However it is advisable to have a medical examination and an exercise test before participation in vigorous intensity exercise (i.e.,  $>60\%$  VO<sub>2</sub>max).

**High risk – individuals classified as High Risk** are those who have one or more signs/symptoms of, or have diagnosed cardiovascular, pulmonary, and/or metabolic disease. The risk of an acute cardiovascular event in this population is increased to the degree that a thorough medical examination should take place and clearance given before initiating physical activity or exercise at any intensity.

## HEALTH HISTORY QUESTIONNAIRE

The purpose of the following questionnaire is to assess your medical risk factors and determining your suitability to serve as a subject in a research study. Should you be retained as a subject, all information from this form will be kept in strict confidence and will be made available only to the members of the research team. This form will remain in your personal file in a locked file cabinet for the duration of the research study. Should you not be retained as a subject, the form will be promptly destroyed.

ID # \_\_\_\_\_ Date \_\_\_\_\_

Height \_\_\_\_\_ Weight \_\_\_\_\_ BP \_\_\_\_\_/\_\_\_\_\_

Physical Injuries and Limitations \_\_\_\_\_

Are you currently pregnant or think you may be pregnant? Y N

Have you ever had any of the following cardiovascular problems? (check all that apply)

Heart Attack/ Myocardial Infarction _____	Heart Surgery _____
Chest Pain or Pressure _____	Swollen Ankles _____
Arrhythmias/Palpatations _____	Heart Murmur _____
Heart Valve Problems _____	Dizziness _____
Shortness of Breath _____	Congestive Heart Failure _____

Have you ever had any of the following? (check all that apply)

Hepatitis/HIV _____	
Rheumatic Fever _____	High Blood Pressure _____
Kidney/Liver Disease _____	Obesity _____
Diabetes (type) _____	Asthma _____

Emphysema	_____	Thyroid Problems	_____
Stroke	_____		
Total Cholesterol >220 mg/dL	_____	HDL <35 mg/dL	_____
LDL Cholesterol >135 mg/dL	_____	Triglycerides >150 mg/dL	_____

Do any immediate blood relatives (biological parent & siblings only) have any of the above conditions? If yes, list the condition, family relation, and the age at diagnosis.

Is your Mother Living? Y N      Age at death\_\_\_\_\_ Cause\_\_\_\_\_

Is your Father Living? Y N      Age at death\_\_\_\_\_ Cause\_\_\_\_\_

Are you currently taking any medications (prescription or over the counter), vitamins, or dietary supplements? Y N

If yes, please list\_\_\_\_\_

\_\_\_\_\_

If you are currently taking medications, vitamins, and/or supplements, are you aware of any that could change the rate or rhythm of your heart? Y N

If yes, please list\_\_\_\_\_

\_\_\_\_\_

Do you now, or have you ever, used tobacco Y N      If yes, what type?\_\_\_\_\_

How Long?\_\_\_\_\_ Amount\_\_\_\_\_/day      Years since quitting?\_\_\_\_\_



How often do you drink the following?

Caffeinated coffee, tea, or soda \_\_\_\_\_oz/day

Hard liquor\_\_\_\_\_oz/wk

Wine\_\_\_\_\_oz/wk

Beer\_\_\_\_\_oz/wk

Do you currently practice regular physical exercise? Y N

If yes, what type\_\_\_\_\_

Frequency of exercise\_\_\_\_\_x/wk

Volume per week\_\_\_\_\_hrs

Exercise Intensity\_\_\_\_\_

What is your current level of emotional stress? High      Moderate      Low

Thank you for your interest in this study. Please adhere to the following requirements for participation in this study. If you have questions or concerns, please contact Dr. Morris @ 828-262-7120.

### Training Requirements

1. Maintain consistent training durations and intensities for the duration of the study.
2. Maintain a consistent training pattern for the duration of the study. For instance, do not change the order of your hard and easy days.
3. Avoid training on the days of your exercise tests and during the 24 hour period leading up to each test. You are allowed no more than 30 minutes of exercise at  $\leq 60\%$  maximum heart rate.
4. Keep a training log for the 24 hours leading up to each test. Bring this log with you when you come in for each test.

### Dietary Requirements

1. If accepted into this study, you must maintain a diet record list of all foods consumed for 24 hours prior to each exercise test. Please bring this record with you when you come in for each exercise test.
2. Do not use any vitamin or mineral supplements for the duration of the study. This includes fatty acid supplements such as fish oil, flaxseed oil, etc. Also avoid consumption of vitamin A,C,&E supplements for the duration of the study.
3. Maintain a consistent diet for the duration of the study, especially during the final 3 days leading up to your exercise tests. A list of foods to avoid is provided.

4. Do not use aspirin, ibuprofen, or compounds containing acetaminophen throughout the study.

5. Please consume 1 liter of fluids the night before and 1 liter for fluids the morning of each exercise test.

6. Please avoid high-fat foods 4 hours prior to each visit

7. You will be given a small snack to be consumed 2 hours prior to arrival. You will only be allowed to consume the small snack and water 2 hours prior to arrival. Upon arrival, you will not be allowed to eat or drink anything other than the treatment fluid until after the exercise test.

8. Avoid alcoholic beverages or caffeine 24 hours prior to each exercise test.

9. Avoid chewing gum or mouthwash 48 hours prior to each exercise test.

10. Avoid consumption of high nitrate containing foods 36 hours prior to each exercise.

Examples:

- processed/ cured meats (ex. hotdogs, lunch meats, bacon, corned beef, ham, smoked meats, and sausages),

- leafy and root vegetables (ex radishes. carrots, any kind of potatoes, beets, turnip, parsnips, dill, broccoli, spinach, cauliflower, any cabbage, lettuce, mustard greens, celery, endive, fennel, kohlrabi, leek, parsley, cress, chervil, rocket (rucola), pumpkin, chicory),

- vegetable juice,

11. Please limit fruits to 2 servings and vegetables to 3 servings/day. Please avoid fruits and vegetables 24 hours prior to exercise. One serving of sliced fruits = ½ cup or one whole piece of fruit. One serving of leafy vegetables = 2 cups; of non-leafy vegetables = 1 cup.

12. Avoid excessive consumption (more than 2 servings/day; 1 serving = 3 oz = deck of cards) of high fat foods throughout the study. Avoid high fat foods 48 hours prior to exercise such as:

- High fat meats such as, beef, pork, and fried meats
- Oils, margarine, butter, gravies, high fat salad dressings
- Meat or cream sauces
- Eggs, regular cheese, whole milk

13. Avoid foods containing garlic 48 hours prior to exercise.

# 24-h Food Record

Name \_\_\_\_\_ Age \_\_\_\_\_

Height \_\_\_\_\_ Weight \_\_\_\_\_ Sex \_\_\_\_\_

Home Phone \_\_\_\_\_ Work Phone \_\_\_\_\_

## **Directions: FOLLOW FOOD LIST REQUIREMENTS.**

1. Please do not alter your dietary intake during the seven-day period leading up to each test. Some people tend to eat better or take in less food than normal while recording, and this decreases the usefulness of the final nutrient intake report. Record everything you eat or drink, including water, juices, condiments, fats, spices, supplements, or medications.

2. Another major problem is inaccurate reporting of the size or weight of food portions eaten. As much as possible, measure everything you eat with a cup, tablespoon, or teaspoon. Food portions can also be weighed (if you have a food scale), or the weight recorded in ounces as given in the food label. When measurement of size or weight is not possible, record the portion size as small, medium, or large.

3. Do not rely on memory: please take this food record with you and list foods immediately after they are eaten. Please print all entries.

4. Please be as specific as possible when describing the food item eaten or fluid consumed: the way it was cooked or prepared, brand name, and if the food was fresh, frozen, smoked, stewed, fried, baked, broiled, raw, braised, or canned.

<i><b>Date (Time food eaten)</b></i>	<i><b>Describe Carefully the Food Item or Fluid Consumed and Method of Preparation</b></i>	<i><b>Amount Eaten</b></i>

# 24-h Physical Activity Log Record

Name \_\_\_\_\_ Age \_\_\_\_\_

Height \_\_\_\_\_ Weight \_\_\_\_\_ Sex \_\_\_\_\_

Home Phone \_\_\_\_\_ Work Phone \_\_\_\_\_

## **Directions: FOLLOW Physical Activity REQUIREMENTS.**

- 1. Please do not alter your physical activity during the seven-day period leading up to each test. Some people tend to change their activity level while recording, and this decreases the usefulness of the final physical activity report. Record every activity you complete included duration, intensity (or perceived intensity), and type of exercise.*
- 2. Do not rely on memory: please take this activity record with you and list activity immediately after it is completed. Please print all entries.*
- 3. Please describe the physical activity completed in the last 24 hours.*

<i>Date</i>	<i>Describe Carefully the type of physical activity</i>	<i>Intensity (or perceived intensity)</i>	<i>Duration (time)</i>

# 24-h Sleep Record

Name \_\_\_\_\_ Age \_\_\_\_\_

Height \_\_\_\_\_ Weight \_\_\_\_\_ Sex \_\_\_\_\_

Home Phone \_\_\_\_\_ Work Phone \_\_\_\_\_

**Directions:** Please come fully rested to each trial. Sleep does affect performance, so please maintain a good quality sleep schedule throughout the study.

1. Please describe the quality and quantity of sleep over the last 24 hours.

<i>Date</i>	<i>Sleep Time (hours and minutes)</i>	<i>Please describe quality of sleep (number of times wake up throughout the night, do you feel rested the next morning?)</i>



# Training History

Name \_\_\_\_\_ Age \_\_\_\_\_

Height \_\_\_\_\_ Weight \_\_\_\_\_ Sex \_\_\_\_\_

Home Phone \_\_\_\_\_ Work Phone \_\_\_\_\_

**Directions:** Please answer the questions pertaining to the last eight weeks to current training status. Please be as specific as possible. Ex. for interval training: work:rest, sets

1. Please describe your training over the past eight weeks.

<i>Dates</i>	<i>Type of exercise</i>	<i>Frequency (how many days a week)</i>	<i>Intensity (or perceived intensity)</i>	<i>Duration (time of each activity)</i>

2. Please describe your competition experiences of the past eight weeks.

<i>Dates</i>	<i>Type of exercise</i>	<i>Event</i>	<i>Competition Level</i>	<i>Results (time)</i>	<i>Was this a typical performance for you</i>

## **Vita**

Jennifer Christine Arms was born in Chapin, South Carolina on September 13, 1988, to Kathryn and Randall Arms. She graduated from Chapin High School in 2006. In the fall of 2006, Jennifer enrolled at Boston University, Sargent College of Rehabilitation and Health Sciences, to pursue a Bachelor of Science degree in Human Physiology/Pre-Medicine. During her four years at Boston University, she was a member of the National Society of Collegiate Scholars and of Sargent Honor Society. She was on the varsity women's ice hockey team all four years and received the NCAA Student Athlete Scholar Award twice. In May 2010, Jennifer graduated from Boston University *summa cum laude*. Following graduation, she spent a year coaching a youth ice hockey travel team and strength training various youth athletes. In the fall of 2011, she pursued a Master of Science degree in Exercise Science with an emphasis in Strength and Conditioning from Appalachian State University, where she also accepted a graduate research assistantship. During her time at Appalachian State University, Jennifer was a member of The Honor Society of Phi Kappa Phi and the Cratis Williams Society of Outstanding Graduates of the Graduate School at Appalachian. Following the achievement of her MS degree in the spring of 2013, she decided to extend her studies by working toward a doctoral degree in physical therapy. Jennifer is attending Duke University with a graduation date of May 2016 in hopes of working in sports physical therapy. Jennifer is a member of the National Strength and Conditioning Association and is a Certified Strength and Conditioning Specialist.